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CYTOCHEMICAL CHANGES OF ACUTE LUPUS ERYTHEMATOSUS

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MORPHOLOGIC investigations of acute lupus erythematosus have been directed primarily at defining it by descriptive characterization of the alterations of organs and tissues. Most attention has been paid in recent years to changes of the extracellular portions of the connective tissue, such as fibrinoid alteration of the collagen fibers and the ground substance [1] and, in the latter, a striking increase of metachromatic material.[2] It has been the aim of subsequent investigations to interpret these morphologic findings in terms of the morbid process. But it was recognized that similar alterations could be observed in heterogeneous situations, both in human disease and under experimental conditions. It became evident that the connective tissue alterations were not adequately defined by mere microscopic analysis. It seemed necessary, therefore, to investigate the nature of the microscopically visible alterations with methods of biochemistry and biophysics. Such explorations have been carried out in this laboratory for the past years but have not yet reached maturation. In the meantime the possibility was not neglected that a careful microscopic examination of new cases might reveal additional structural characteristics.

It was our good fortune to encounter, in the past year 4 cases of acute lupus erythematosus which showed certain tissue changes in so

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1. Klemperer, P.; Pollack, A. D., and Baehr, G.: Arch. Path. **37**:569, 1941.

2. Klemperer, P.: Ann. Int. Med. **28**:1, 1948.

striking a manner that their importance was obvious. Identical changes had already been described in previous years, but no attempt had been made to interpret their meaning.

HISTORICAL REVIEW

In 1932 Gross [3a] observed "clumps and packets of hematoxylin-stained bodies which on the whole appear to be spindle shaped" in the valvular vegetations of Libman-Sacks disease. This disease was subsequently identified [3b] with acute lupus erythematosus. He expressed the belief that the hematoxylin-stained bodies were "undoubtedly somewhat pycnotic and karyorrhectic nuclear masses" and stated subsequently that "thus far they have been encountered only in this disease" [namely, acute lupus erythematosus].

Ginzler and Fox [4] were the first to report the presence of similar large hematoxylin-staining aggregates in necrotic lymph nodes in a case of acute lupus erythematosus. Previous authors had referred briefly to necrotic foci of lymph nodes and spleen without detailed description. In describing the microscopic appearance of the kidneys of their case Ginzler and Fox referred to pinkish violet-staining granular degeneration and necrosis of the endothelial cells of the glomerular capillaries. Klemperer, Pollack and Baehr [1] also referred to basophilic debris in focal loop necrosis which they found in 17 of 20 cases of acute lupus erythematosus. Rakow and Taylor [5] observed similar glomerular lesions, as indicated by their description and illustrations. Foldes' [6] case 1 also showed identical glomerular alterations.

Such changes were most conspicuous in three of our recent observations. In addition we noticed similar, though less striking, hematoxylin-stained bodies in several sections from other parts of the body. These findings led us to reexamine our collected cases of acute lupus erythematosus of the past twenty years in order to obtain information regarding the frequency and the distribution of the hematoxylin-stained bodies.

MATERIALS AND METHODS

This report concerns 35 cases of acute systemic lupus erythematosus. All cases which had come to autopsy with the clinical diagnosis since 1928 were included, with the exception of 3. Two cases in which the clinical diagnosis had been placed in doubt by atypical changes observed at necropsy, and 1, an undoubted case, in which the changes to be discussed were present but obscured by diffuse calcinosis, were omitted. Some of these cases have formed the material for previous reports.

3. Gross, L.: (*a*) Contributions to the Medical Sciences in Honor of Emanuel Libman, by His Pupils, Friends and Colleagues, New York, International Press, 1932, vol. 2, p. 527; (*b*) Am. J. Path. **16**:375, 1940.

4. Ginzler, A. M., and Fox, T. T.: Arch. Int. Med. **65**:26, 1940.

5. Rakow, H. L., and Taylor, J. S.: Arch. Int. Med. **88**:70, 1942.

6. Foldes, J.: Am. J. Clin. Path. **16**:160, 1946.

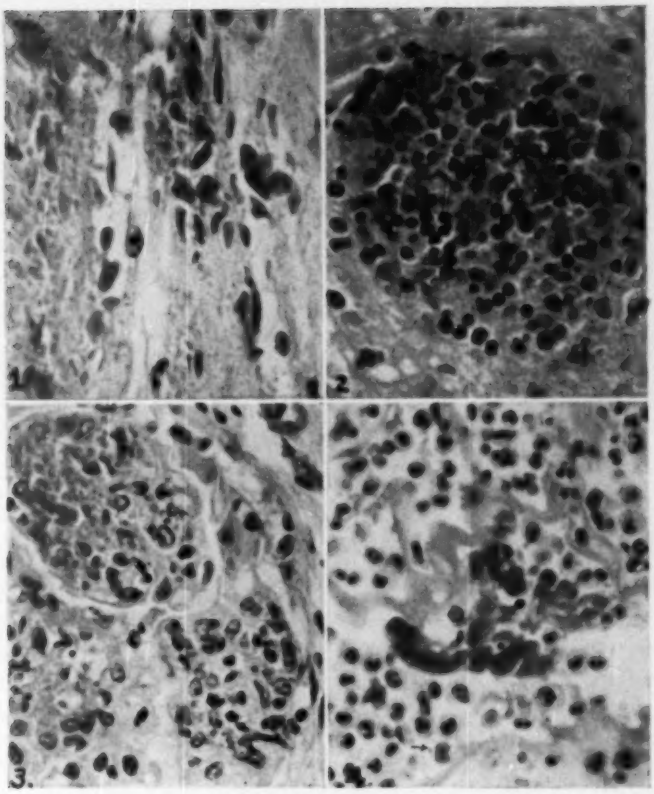
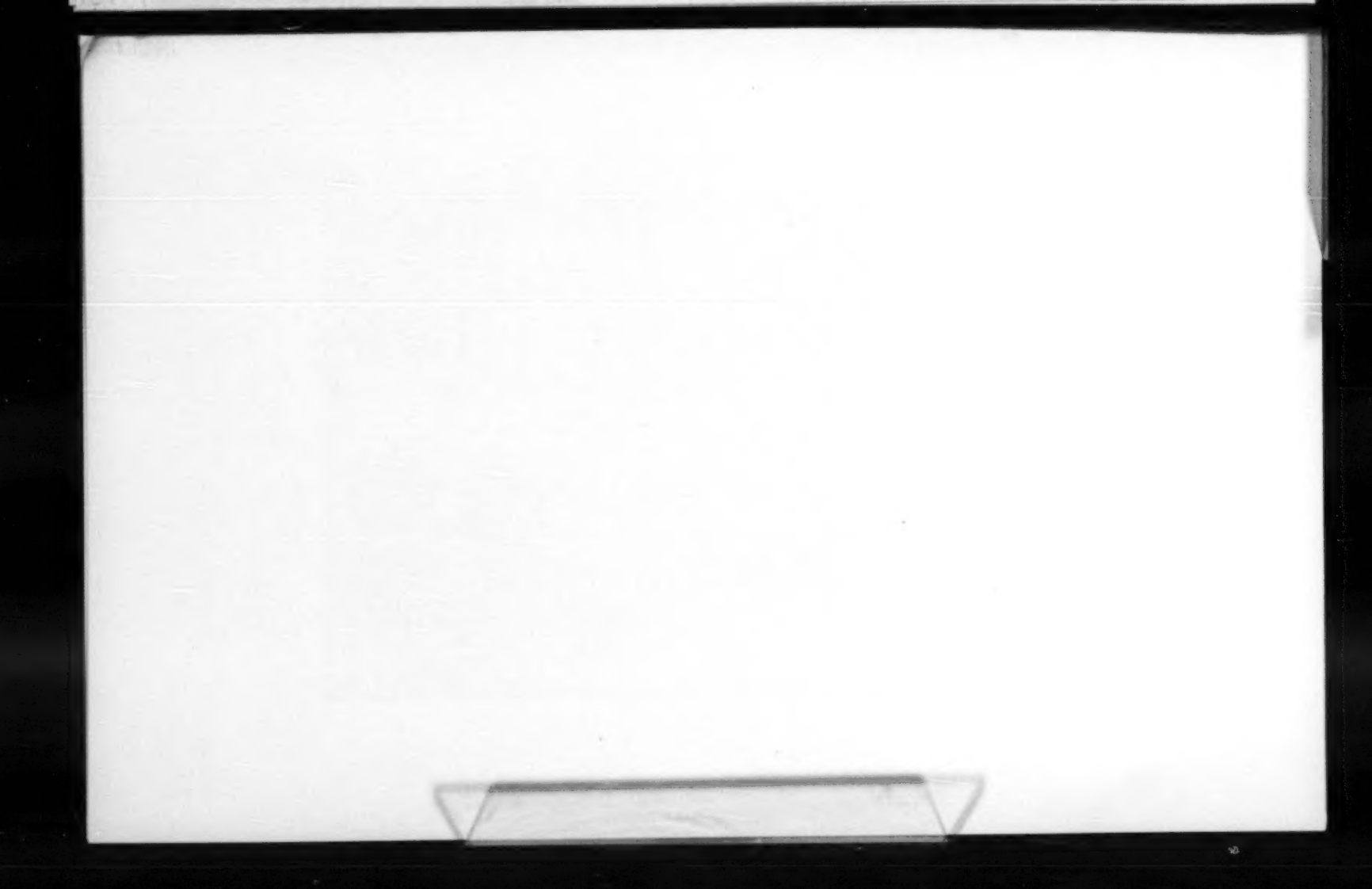


Fig. 1.—Section of grossly normal mitral valve showing numerous spindle-shaped hematoxylin-stained bodies, without surrounding cytoplasm. (× 441.)

Fig. 2.—Area in biopsy lymph node showing scattered hematoxylin-stained bodies with homogenization and purple staining of lymphocytic nuclei. (× 441.)

Fig. 3.—Glomerulus showing numerous purple-stained hematoxylin-staining bodies. (× 441.)

Fig. 4.—Pleura showing small aggregation of hematoxylin-stained bodies and one histiocyte (arrow), with nuclear homogenization and purple staining. (× 441.)



In all cases hematoxylin and eosin-stained thin paraffin sections of material fixed in 20 per cent formaldehyde solution were available. For the demonstration of the changes to be described such material was more desirable than that fixed in Zenker's solution. In the 4 recent cases tissue was fixed in addition in 95 per cent alcohol and Carnoy's acetic acid–absolute alcohol (1:3).

A variety of stains for connective tissue, fibrin, bacteria, mucus and calcium were applied to selected sections. But for the demonstration of the cellular alterations to be described we depended entirely on hematoxylin-eosin stains. For the cytochemical analysis, the Feulgen reaction (always controlled by omission of the acid hydrolysis) and methyl green in the modification of Pollister and Leuchtenberger,[7] and methyl green–pyronine (Unna-Pappenheim) were utilized. These methods were primarily applied to material fixed in Carnoy's solution. However, material fixed in 20 per cent formaldehyde solution and Zenker's solution also gave satisfactory results. Certain sections were incubated with ribonuclease after the method of Stowell and Zorzoli.[8] Ultraviolet ray photography was done with Zeiss equipment of the type described by Koehler[9] in 1904. The light source was a Hanovia SC 2537 mercury vapor lamp.

Photometric studies or light absorption, according to Pollister and Ris[10] and Pollister and Leuchtenberger,[7] were done on lymph node sections stained with the Feulgen reaction and methyl green. The photometric device was similar to one which has been described by Pollister and Moses.[11] In the present investigation a Wratten 26 filter, instead of an interference filter, was used for isolation of the red light for absorption studies of methyl green. This gives somewhat higher Feulgen–methyl green ratios.

DESCRIPTION OF LESIONS

Endocardium.—The fully developed "hematoxylin-stained bodies" of Libman-Sacks endocarditis have been well described by Gross.[5] Such lesions were found in 7 of our cases, representing all those in which the Libman-Sacks type of atypical coarse verrucous endocarditis was observed grossly. Microscopically, the vegetations consist of a finely granular eosinophilic matrix in which are embedded "clumps and packets" of ovoid or spindle-shaped purple-blue structureless bodies, each about the size of a cell. In some areas these bodies coalesce to form large masses (fig. 5). Scattered diffusely throughout the eosinophilic matrix of the vegetation one finds polymorphonuclear leukocytes and mononuclear cells of histiocytic appearance. Many of these cells show bizarre nuclei. In addition, one notices that many nuclei of polymorphonuclear leukocytes, as well as of histiocytes, stain purple in the same tint as that of the hematoxylin-stained bodies. Moreover, the

7. Pollister, A. W., and Leuchtenberger, C.: Proc. Nat. Acad. Sc. **35**:111, 1949.

8. Stowell, R. E., and Zorzoli, A.: Stain Technol. **22**:51, 1947.

9. Koehler, A.: Ztschr. f. wissenschaft. Mikrobiol. **21**:272, 1904.

10. Pollister, A. W., and Ris, H. R.: Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1947, vol. 12, p. 147.

11. Pollister, A. W., and Moses, M. J.: J. Gen. Physiol. **32**:567, 1949.

nuclei of such cells appear homogeneous, with loss of chromatin structure. Often one finds such nuclear changes in cells adjacent to the amorphous hematoxylin-stained bodies. These nuclear changes, i. e., homogenization and purple staining, can easily be distinguished from the well known features of nuclear pyknosis or karyolysis. However, cells showing the latter alteration are also found, but they are far less frequent. Occasionally, polymorphonuclear leukocytes, as well as histiocytes, are seen with normal nuclei but with cytoplasmic inclusions of nuclear material of the same structural and tinctorial characteristics as the free hematoxylin-stained bodies. The vegetation merges imperceptibly with the underlying valve or mural endocardium, which is thickened, edematous and moderately cellular. Foci of fibrinoid alteration of collagen may be found. The infiltrating cells are mainly histiocytes, lymphocytes and plasma cells intermingled with many proliferating fibroblasts and Anitschkow myocytes.

In addition to the conspicuous aggregates within the valvular vegetations, hematoxylin-stained bodies are found in smaller collections or even scattered singly within valve substance (fig. 1), ring or mural endocardium. They were observed in 21 of our 35 cases. The bodies vary in size from that of a nuclear fragment to that of several cells. They are entirely without structure, but many are of the size and shape of fibroblasts or histiocytes. All stages of transition can be observed. Intermediate forms are seen with smudgy nucleus, loss of chromatin network and basophilic cytoplasm.

Whenever alterations of collagen (swelling, fragmentation and increased eosinophilia) are associated with inflammatory reaction, the polymorphonuclear leukocytes, lymphocytes and histiocytes frequently show this characteristic nuclear alteration.

Lymph Nodes and Spleen.—Large hematoxylin-staining bodies of the type described by Ginzler and Fox [4] were found in 4 of our cases. These are amorphous masses with smooth outline occurring in and around completely necrotic areas, sometimes within sinuses (fig. 6), staining deep purple-blue. In the lymph nodes in these 4 cases and in 7 others, and in necrotic areas in the spleen in 6 cases, less conspicuous but similar changes have been found. These consist of collections of purple-staining bodies smaller than any cell, others of about lymphocytic size and still others which are larger, formed presumably by confluence of smaller bodies. Transition forms can be seen leading from apparently normal lymphocytes through lymphocytes with smudged nuclei to free hematoxylin-staining bodies. Other types of nuclear disintegration, such as pyknosis and karyorrhexis, are present. An identical picture was observed in a lymph node removed during the life of the patient (fig. 2)

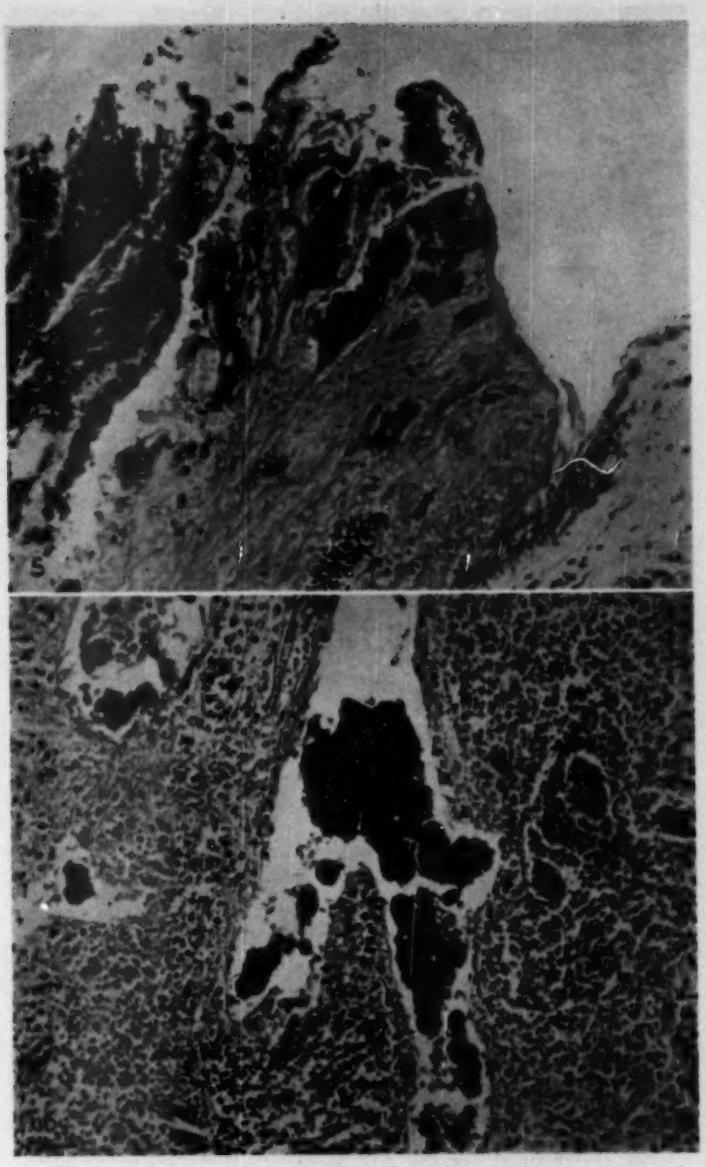


Fig. 5.—Coarse verrucous endocarditis. Vegetation with large aggregates of hematoxylin-stained bodies. (× 120.)

Fig. 6.—Large aggregates of hematoxylin-stained bodies in a sinus of a necrotic lymph node. (× 120.)

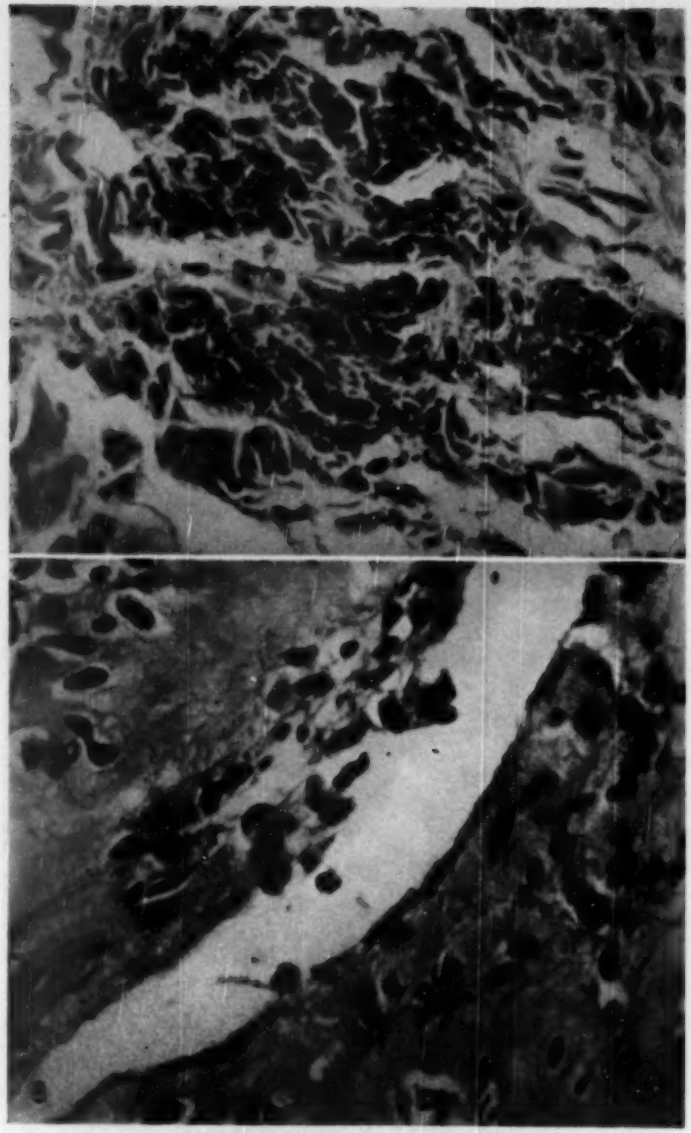


Fig. 7.—Synovia of knee joint showing scattered hematoxylin-stained bodies. Note the spindle-shaped dark clumps resembling cellular nuclei but without surrounding cytoplasm. (× 600.)

Fig. 8.—Ovary with isolated hematoxylin-stained bodies at the upper right, in the wall of the vein. Note the contrast between these and the normal nuclei at the upper left. (× 600.)

Kidneys.—Hematoxylin bodies were found in 27 of our 35 cases. They are encountered mostly within the lumens of glomerular capillaries (fig. 3), occasionally within the walls of interlobular arteries and rarely within the stroma. The bodies may be found in nearly every glomerulus; sometimes, however, they are scarce and can be found only after a long search. They are often associated with swelling and disintegration of the capillary basement membrane. The origin of these bodies is not easily ascertained; some seem to originate from circulating

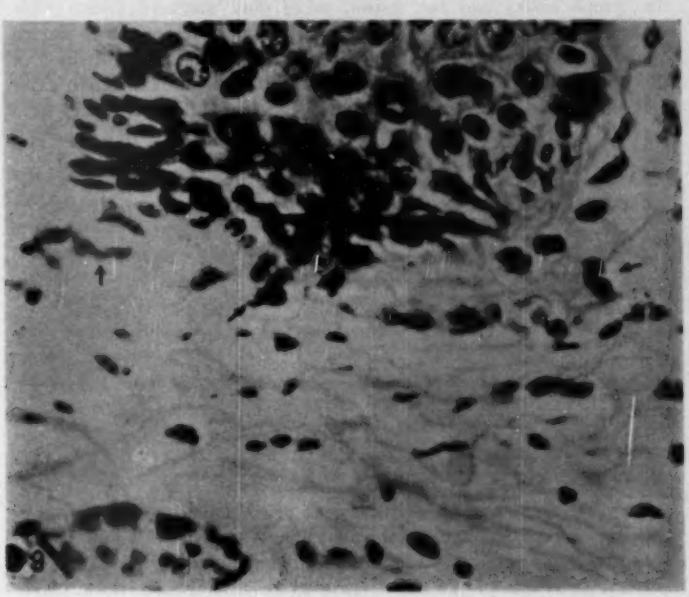


Fig. 9.—Vagina. Hematoxylin-stained bodies in subepithelial stroma. Note that epithelial nuclei show clear chromatin network. (× 600.)

polymorphonuclear leukocytes. Nuclear alterations of the endothelial or visceral epithelial cells cannot definitely be established.

Other Sites.—In serosal membranes (5 cases), synovia (3 cases), skin (3 cases), mucous membranes (2 cases), vessels (4 cases), mediastinal tissues (2 cases), bone marrow (1 case), liver (1 case), pancreas (1 case), myocardium (2 cases) and ovaries (13 cases) identical bodies have been noted. See figures 4 (serosal membrane of pleura), 7 (synovia), 8 (ovaries) and 9 (mucous membranes of vagina). The origin of the bodies could be traced to fibroblasts, histiocytes,

endothelial cells (Kupffer cells included), lymphocytes and polymorphonuclear leukocytes. In no case were cells of other than mesenchymal origin definitely implicated.

COMMENT

Table 1 summarizes the frequency and distribution of hematoxylin bodies in 35 cases of acute lupus erythematous. It will be noted that in this series the kidneys and the endocardium were by far the most frequent sites. Lesions were observed with somewhat lower frequency in the lymph nodes and the spleen, while only scattered bodies were observed in other organs. This may or may not present a true picture of the organic incidence of these lesions. For many years, attention in this laboratory has been focused on the heart and the kidneys in cases of acute lupus erythematosus. Multiple sections of these organs were available in every case (except 1 in which by oversight no kidney sections were taken at autopsy). Only the usual single routine sections of grossly uninvolved organs were available in many cases, and certain sites could only rarely be examined because of limitations of the autopsy permit. For example, in only 5 cases were sections of synovia taken. Hematoxylin-staining bodies were found in 3 of these.

TABLE 1.—*Incidence of Nuclear Change in 35 Cases of Systemic Lupus Erythematosus*

Organ Involvement	Cases
Glomeruli	27 (of 34)
Endocardium (mural and valvular)	21
Lymph node	11
Spleen	6
Other mesenchymal tissue	14
Generalization	
2 or more organs involved	24
1 organ only involved (kidney in 5, endocardium in 3)	8
Total Incidence	
Present in	32
Absent in	3

In all, 32 of our 35 cases showed hematoxylin-stained bodies in one or more organs (table 1). In 1 of the 3 cases in which they were not observed, Libman-Sacks endocarditis was reported at autopsy. Sections of the involved tissue were taken and described at the time but have disappeared from our files. We found no other hematoxylin-stained bodies throughout the case, although other microscopic changes of acute lupus erythematosus, including "wire loop" glomerular capillaries and splenic periarterial fibrosis, were present. The second and third cases in which the hematoxylin-staining bodies were not seen presented unequivocal changes in the extracellular portions of the connective tissue.

We were able in 24 of the 32 cases in which the bodies were observed to demonstrate dissemination of the lesions, i. e., their occurrence at two or more different sites. In 6 of these 24 cases involvement was extremely widespread. With more complete material, further evidence of generalization probably could be obtained. In this respect it is noteworthy that in each of our last 4 cases involvement of several sites was observed.

Since the endocardium and the kidneys had been found most frequently affected, sections of these organs were selected for the study of control material. Careful search of many sections from random autopsies has failed to reveal changes similar to those described. In addition, cases of rheumatic and bacterial endocarditis, of acute and chronic diffuse glomerulonephritis, of "embolic" focal glomerulonephritis and of polyarteritis nodosa were studied. In none were free hematoxylin-staining bodies observed. Only in 1 case of diffuse scleroderma were such bodies seen in the glomeruli. This was a case, reported by Pollack,[12] in which conspicuous "wire loop" glomerular capillaries and fibrinoid alteration of the endocardium had been observed, changes identical with those encountered in acute lupus erythematosus.

The fact that the hematoxylin-staining bodies were not found in a large control material justifies the conclusion that they must not be regarded as trivial postmortem changes. This conclusion is supported by the fact that identical bodies were found in a lymph node removed during the life of a patient.

In necrotic areas of vegetations of acute bacterial endocarditis, in occasional necrotic walls of blood vessels in cases of polyarteritis nodosa, sometimes in necrotic carcinomatous lymph nodes and in other sites where massive necrosis of tissue is a factor, peculiar nuclear changes are sometimes seen in individual cells. These cannot be distinguished from the nuclear alterations which we have described as initial stages leading to the structures described as free hematoxylin-stained bodies. In all these instances, however, two major (and, we believe, fundamental) points of difference arise. First, in all the situations mentioned there are seen severe degenerative cellular alterations characterized by pyknosis, karyolysis and karyorrhexis—and these conventional necrobiotic changes are the predominant ones and are always accompanied by a severe inflammatory response. In the lesions which we have observed in lupus, however, the conventional types of nuclear alterations are much less conspicuous whenever the hematoxylin-stained bodies are most massive, and absent whenever these bodies are collected in small groups or scattered singly throughout the tissue. Second, whenever lesions which simulate the hematoxylin-stained bodies are found in conditions other than lupus, they are easily recognized as nuclei within

12. Pollack, A. D.: Arch. Path. **29**:859, 1940.

the cells. In other words, in lupus alone the free hematoxylin-stained bodies are seen, and agglomeration of these bodies in large masses occurs only in this disease.

For these reasons we feel justified in concluding that the free hematoxylin-staining bodies are a specific structural criterion of lupus erythematosus and that they originate in an alteration of the nuclei of mesenchymal cells which is characteristic for lupus erythematosus.

It now becomes incumbent on us to define the nature of this peculiar cellular alteration. Ginzler and Fox had already ruled out, by means of the Von Kossa stain, the possibility that calcium was present in the masses within the lymph nodes. Gross ruled out the possibility

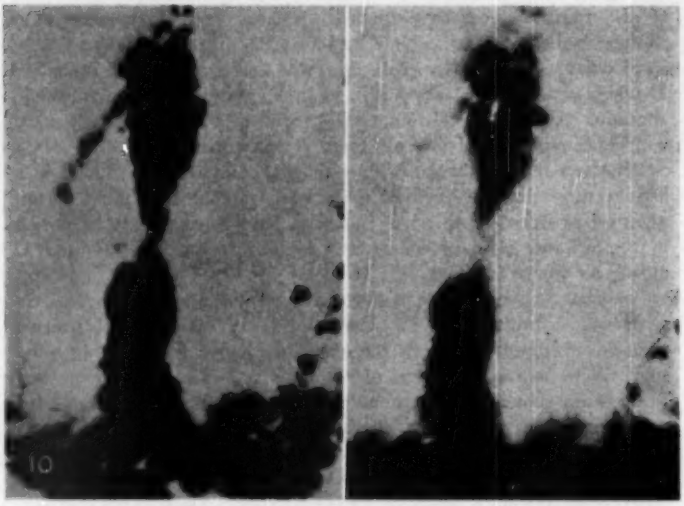


Fig. 10.—Feulgen reaction, left, and ultraviolet ray (2,537 angstroms) photograph, right, of the same aggregation of hematoxylin-stained bodies in the mitral valve. (× 600.)

that bacteria were harbored within the valvular vegetation, by Gram and other bacterial stains. Our own investigations of hematoxylin-stained bodies in the same and other sites were confirmatory. Gross [38] had already concluded that they were of nuclear origin, and our own studies of hematoxylin-eosin sections seemed to leave no doubt that this interpretation was correct. However, it appeared to us necessary to prove by direct cytochemical tests an interpretation formerly based on structural features alone.

Sections of endocardial vegetation and of lymph nodes with large aggregates of hematoxylin-stained bodies, as well as of kidneys and of

synovial tissues, were therefore examined. The results were identical in every sample and can therefore be summarized:

A controlled Feulgen reaction stains the bodies brilliant red. They show very strong absorption of ultraviolet rays at 2,537 angstroms (fig. 10). If treated with hot trichloroacetic acid, the bodies lose the ability to absorb ultraviolet rays of the same wavelength and become Feulgen negative.

The results of these two tests prove that the hematoxylin-staining bodies contain desoxyribose nucleic acid.

Methyl green used in the modification of Pollister and Leuchtenberger [7] stains the bodies faintly green. Duplicate photometric studies

TABLE 2.—*Methyl Green and Feulgen Extinction Values of Lymph Node Aggregates of Hematoxylin-Stained Bodies and of Lymphocytes*

Aggregate Number	Methyl Green E 625	Feulgen E 550	Ratio Feulgen to Methyl Green	Nucleus Number	Methyl Green E 625	Feulgen E 550	Ratio Feulgen to Methyl Green
1	.377	.854	2:1	1	.319	.538	1.7:1
2	.189	1.60	8.5:1	2	.319	.538	1.7:1
3	.260	1.087	4.2:1	3	.409	.565	1.4:1
4	.260	.806	3.4:1	4	.495	.569	1.1:1
5	.356	1.087	2.9:1	5	.351	.481	1.3:1
6	.337	1.046	2.9:1	6	.330	.381	1.1:1
7	.269	1.377	4.9:1	7	.301	.367	1.2:1
8	.319	1.696	5.9:1	8	.301	.409	1.3:1
9	.244	.884	3.6:1	9	.306	.395	1.3:1
10	.215	1.006	4.9:1				
11	.174	1.528	8.7:1				
12	.272	1.256	6.3:1				
13	.356	1.596	3.3:1				
14	.232	.820	3.6:1				
15	.292	.770	2.6:1				
16	.143	.950	6.7:1				
17	.135	.920	6:1				
18	.102	.745	7.3:1				
19	.108	.854	5:1				

E 625 and E 550 indicate extinctions at 625 and 550 millimicrons.

of 20 large aggregates of lymph node hematoxylin-staining bodies were carried out as follows: The slides were first stained with methyl green and the extinction measured in red light (625 millimicrons); they were then decolorized and stained by the Feulgen method, and the identical areas measured by green light (550 millimicrons). From these two measurements, the Feulgen–methyl green extinction ratio could be computed for one or more small areas of each aggregate. Twenty sets of readings are shown in table 2. As can be seen, the ratios ranged from 2.6:1 to 8.7:1. Lymphocytic nuclei, apparently normal, from the vicinity of the aggregate were measured in the same way. The results are shown on the right in table 2. The ratios were fairly uniform,

ranging from 1.1 : 1 to 1.7 : 1 and in no case approaching the lower ratio of the aggregates. These lymphocyte ratios are similar to those found in tissues known to be normal.

It has been pointed out by Pollister and Leuchtenberger [7] that the methyl green stain may be considered a delicate indicator of the state of polymerization of the desoxyribose nucleic acid molecule. Any lowering of the degree of polymerization results in decrease of the amount of methyl green which the nucleic acid binds. The Feulgen reaction, on the contrary, appears to be unaffected by these slight changes in the physical state of the desoxyribose nucleic acid. In normal nuclei of all types the extinction ratio of these two dyes is nearly constant. With the method of measurement used in this study, the normal ratio is close to that given for the nine nuclei of table 2. The abnormally high Feulgen–methyl green ratios of the hematoxylin-staining bodies are to be interpreted as an indication that the desoxyribose nucleic acid is in all cases more or less depolymerized. This physical state of the desoxyribose nucleic acid may account for the appearance of the large masses in endocardial vegetations and lymph nodes as due to the confluence of the less viscous depolymerized nucleic acid.

Kurnick [13] and Friedman [14] have demonstrated in vitro that pure desoxyribose nucleic acid loses its stainability with methyl green after depolymerization by desoxyribose nuclease or by acid hydrolysis. They found that it then stains with pyronine. Since ribose nucleic acid also stains with pyronine, Kurnick ascribed the pyroninophilia of depolymerized desoxyribose nucleic acid to the decrease in molecular size. It has been shown, however, that pyronine is not a dye specific for nucleic acid but one that reacts also with other substances—for instance, colloid of the thyroid gland.[7] The hematoxylin-staining bodies stain red with the Unna-Pappenheim methyl green–pyronine mixture, and this staining reaction does not appear to be affected if sections are treated with ribonuclease. This test, then, indicates that the hematoxylin-staining bodies do not contain ribose nucleic acid. The pyroninophilia, however, should not be ascribed to the depolymerized state of the desoxyribose nucleic acid, since it may well be due to the protein content of the hematoxylin-staining bodies. Preliminary examinations have shown that the bodies give a strongly positive reaction in Millon's test.

Depolymerization of desoxyribose nucleic acid has recently been observed by C. Leuchtenberger [15] in transplants of sarcoma 180 and normal liver tissue in mice. Leuchtenberger demonstrated that progressive pyknotic alteration of nuclei is associated with progressive depolymerization. This physical alteration of the nucleic acids precedes

13. Kurnick, N.: Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1947, vol. 12, p. 141.
14. Friedman, M.: Personal communication to the authors.
15. Leuchtenberger, C.: Chromosoma, to be published.

their complete break-up, which becomes microscopically manifest in a complete loss of stainability of the nuclear chromatin. Leuchtenberger inferred that the depolymerization in pyknosis is engendered by anoxia. The microscopic appearance of depolymerization of the nuclei of mesenchymal cells in acute lupus erythematosus differs from that shown in Leuchtenberger's preparation in that, with the exception of lymph nodes, pyknosis is not evident. In fact, the nuclei become paler than normal. This may indicate that the manner in which the depolymerization is initiated differs from that of anoxia. This contention is supported by the fact that in anoxic necrosis as it is observed in splenic infarcts, pyknosis is always observed and that only an occasional cell nucleus shows the chromatin homogenization and fading which we observed as a rule in acute lupus erythematosus. The fact that this type of nuclear alteration occurs in single cells, with the majority of surrounding cells showing no changes, also speaks against anoxia as the causative factor. The manner in which depolymerization is initiated in lupus erythematosus is, of course, obscure because the complicated mechanism of normal nucleic acid metabolism is hardly understood. Zamenhof and Chargaff [16] have indicated that in yeast the desoxyribose nucleic acid metabolism is under the control of a finely adjusted balance of depolymerase and an inhibitor of this enzyme. Disintegration of the nucleic acid molecule could be provoked by a potentiation of the depolymerase or by inactivation of the inhibitor; of course, anoxia may be an initiating factor in the disturbance of the normal equilibrium. But other factors not yet understood must also be considered. In this connection, attention should be called to the observations of R. Leuchtenberger.[17] In necrotic mammary carcinoma of mice treated by repeated intravenous injections of triglutamic acid, large hematoxylin-stained masses were found, similar to those seen in necrotic lymph nodes in acute lupus erythematosus. These masses gave a positive reaction in the Feulgen test. No studies were made with methyl green, and no definite conclusions were drawn as to the mode in which the cell necrosis was engendered. However, the author did not think that the destruction of the tumor cells was the result of a primary alteration of the circulation, holding rather that it was brought about by a primary interference with enzymatic activity within the tumor cells. We are inclined to believe that the mechanism of the depolymerization of desoxyribose nucleic acid occurring in the mesenchymal cells in acute lupus erythematosus may be considered in a similar way.

Our investigations, as well as the earlier ones of Gross [3] and Ginzler and Fox,[4] were concerned with peculiar alterations in postmortem

16. Zamenhof, S., and Chargaff, E.: Science **106**:628, 1948.

17. Leuchtenberger, R.: Approaches to Tumor Chemotherapy, Washington, D. C., American Association for the Advancement of Science, 1947, p. 139.

material. We have seen, in addition, identical changes in a biopsy of a lymph node. It seems to us of interest that in recent years Hargraves and associates,[18] Haserick and Sundberg [19] and Sundberg and Lick [20] have reported finding peculiar cells in bone marrow aspirates and blood from patients with acute lupus erythematous. These cells are polymorphonuclear leukocytes containing cytoplasmic inclusions which take nuclear stains. The inclusions are homogenous and are about the size and shape of lymphocytic nuclei. They are reported as giving a positive reaction in the Feulgen test and are considered as evidence of phagocytosis of free nuclear material. The authors considered these cells specific for acute lupus erythematosus, since they were not observed in any other disease. We have observed phagocytosis of the hematoxylin-staining bodies in our tissue sections, both in histiocytes and in polymorphonuclear leukocytes. The similarity of the latter cells and those observed by Hargraves and associates in bone marrow aspirates and designated by them as "L. E." cells is striking.

The microscopic observations here reported add a structural criterion for the anatomic diagnosis of acute lupus erythematosus. Beyond that the cytochemical analysis throws some light on the pathogenesis of this puzzling disease.

SUMMARY AND CONCLUSIONS

Hematoxylin-staining bodies originally described by Gross [3] as observed in the endocardium and by Ginzler and Fox [4] as observed in lymph nodes and kidneys in cases of acute lupus erythematosus have been observed by us in 32 of 35 cases of this disease, often widely distributed throughout the body.

Their origin has been traced to a characteristic alteration of the nuclei of mesenchymal cells.

By cytochemical methods, the free hematoxylin-staining bodies have been determined to contain partially depolymerized desoxyribose nucleic acid.

The nature of the disturbance of the nucleic acid metabolism of mesenchymal cells has been discussed. It has been assumed that depolymerization of the desoxyribose nucleic acid is the result of a primary enzymatic deviation in the finely adjusted metabolism of this compound rather than the result of anoxia, which may also provoke depolymerization.

Attention is called to the similarity of appearance between Hargrave's lupus erythematosus cells in bone marrow aspirates and the free hematoxylin-staining bodies.

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18. Hargraves, M. M.; Richmond, H., and Morton, R. J.: Proc. Staff Meet., Mayo Clin. **23**:25, 1948.
19. Haserick, J. R., and Sundberg, R. D.: J. Invest. Dermat. **11**:209, 1948.
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THORACIC VENOUS ANOMALIES

I. Vascular Connection of the Left Atrium and the Left Innominate Vein (Levoatriocardinal Vein) Associated with Mitral Atresia and Premature Closure of the Foramen Ovale (Case 1)

II. Pulmonary Veins Draining Wholly into the Ductus Venosus (Case 2)

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AS JUDGED from the literature, intrathoracic venous anomalies other than those of the vena cava system are extremely unusual. It is the purpose of this paper to record 2 cases of unusual intrathoracic venous anomalies, to review similar reported cases and to discuss the developmental basis of the vascular malformations. In each of the 2 cases to be reported the subject was a newborn infant. In 1 there was congenital mitral atresia associated with premature closure of the foramen ovale and an anomalous vein which connected the left atrium with the left innominate vein. In the second case all the pulmonary veins failed to connect with the left atrium. Instead, they were joined to form a common venous trunk which entered the abdomen and terminated by joining the ductus venosus.

CASE 1

A 21 day old girl was admitted to the hospital, June 27, 1949, because of cyanosis, which had been present since the day after birth. The birth had been normal, following an uneventful pregnancy. Slight cyanosis had been noted at all times, becoming more intense with crying. Physical examination revealed an acutely ill infant with rapid respirations and moderate cyanosis. The lungs were normal. No cardiac murmurs were audible.

The following laboratory data were obtained. Urinalysis revealed albuminuria, grade 2 (on the basis of 1 to 4, in which 1 designates the mildest and 4 the most severe condition). The value for hemoglobin was 16.2 Gm. in 100 cc. of blood. Erythrocytes numbered 4,870,000 and leukocytes 12,900 per cubic millimeter of blood. Roentgenologic examination demonstrated cardiac enlargement and pulmonary congestion (fig. 1). The electrocardiogram is reproduced in figure 2.

While in the hospital the infant became progressively more dyspneic and cyanotic even though oxygen was administered constantly. A basal systolic heart murmur developed, and pulmonary congestion increased. Death occurred six days after the infant was admitted.

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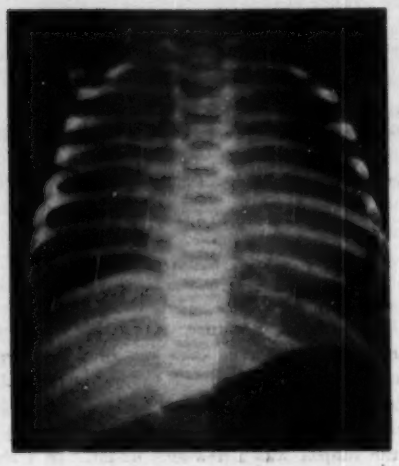
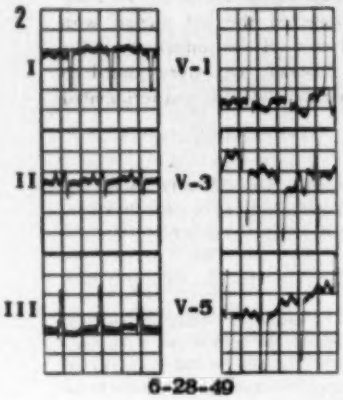




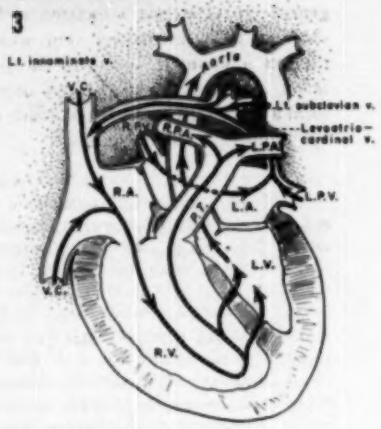


Fig. 1 (case 1).—The roentgenogram of the thorax.

Fig. 2 (case 1).—The electrocardiogram.

Fig. 3 (case 1).—The intracardiac circulation in a case of mitral atresia, premature closure of the foramen ovale and vascular connection of the left atrium and the left innominate vein (levoatriocardinal vein). *L. A.* is the left atrium; *L. P. A.*, the left pulmonary artery; *L. P. V.*, the left pulmonary vein; *L. V.*, the left ventricle; *P. T.*, the pulmonary trunk; *R. A.*, the right atrium; *R. P. A.*, the right pulmonary artery; *R. P. V.*, the right pulmonary vein; *R. V.*, the right ventricle; *V. C.*, the vena cava.

The clinical diagnosis was congenital cardiac disease of a cyanotic type. The possibility of mitral or aortic atresia with progressive closure of the foramen ovale or the ductus arteriosus was considered, since the heart was evidently enlarging and pulmonary congestion was increasing.

Pathologic Features.—The essential pathologic findings were in the heart, the lungs and the great veins. Externally the great arteries appeared to be normally interrelated, although the aorta was somewhat narrower than the pulmonary trunk. There was no transposition. The superior and inferior venae cavae and the coronary sinus communicated in a normal manner with the right atrium. The pulmonary veins, four in number, communicated with the left atrium. The left atrial cavity was diminutive, being about one-half the anticipated normal. The endocardium of the left atrium was gray and opaque. Microscopically it was shown to be thickened by elastic tissue. The mitral valve was atretic (figs. 3 and 4). At the expected location of the mitral orifice there was a depression but there were no elements representing mitral valvular tissue.

Next of interest was the atrial septum. This showed a normally formed foramen ovale with a well defined, raised limbus (fig. 5). The foramen was closed by a membrane which appeared as though in the septum primum there had never developed a secondary opening during embryonic life or, if it had, as though this had closed. As a result, the atrial septum was an intact membrane, revealing no channel by which the two atriums might communicate.

An anomalous vessel of considerable interest was discovered to run between the left atrium and the left innominate vein. This vessel communicated with the left atrium at its left superior wall just medial to the entrance of the left superior pulmonary vein (fig. 6). The vessel measured about 2.5 mm. in diameter. Its course was vertically upward from the superior aspect of the left atrium. In this vertical course it lay anterior to the left bronchus, posterior to the left pulmonary artery and lateral to the ductus arteriosus (fig. 7). Continuing upward, the vessel then coursed in an oblique direction to the right to enter the inferior aspect of the left innominate vein at the level of formation of the left innominate vein by the union of the left internal jugular and left subclavian veins.

The left innominate vein coursed normally to the right along the superior aspect of the thymus and joined the right innominate vein to form the superior vena cava in a normal manner (fig. 8). The right atrial cavity was considerably enlarged (fig. 5). This chamber communicated with the right ventricle by means of a fairly wide tricuspid orifice, 4.7 cm. in circumference. The tricuspid leaflets showed multiple gray translucent elevations, each measuring about 1 mm. in diameter and protruding from the atrial surfaces. The right ventricle was enlarged, presenting a prominent angle at the junction of its superior and right margins. The right ventricular wall measured from 5 to 8 mm. in thickness. There was considerable trabeculation at the areas of union of the papillary muscle and the right ventricular wall. A vertical band of muscle measuring 6 mm. in diameter extended from the ventricular septum in the subpulmonary region to the anterior wall of the upper part of the right ventricle. The pulmonary valve had three leaflets, one anterior, a right and a left, which appeared normally developed. The pulmonary trunk divided normally into its two main branches. The ductus arteriosus had a thick wall and admitted a fine probe. Its appearance was not incompatible with a normally closing ductus. The left ventricular cavity had approximately one quarter of the capacity of the right. Its wall was thickened out of proportion to the size of its cavity. It measured 6 mm. in thickness. The left ventricle communicated normally with the aorta.

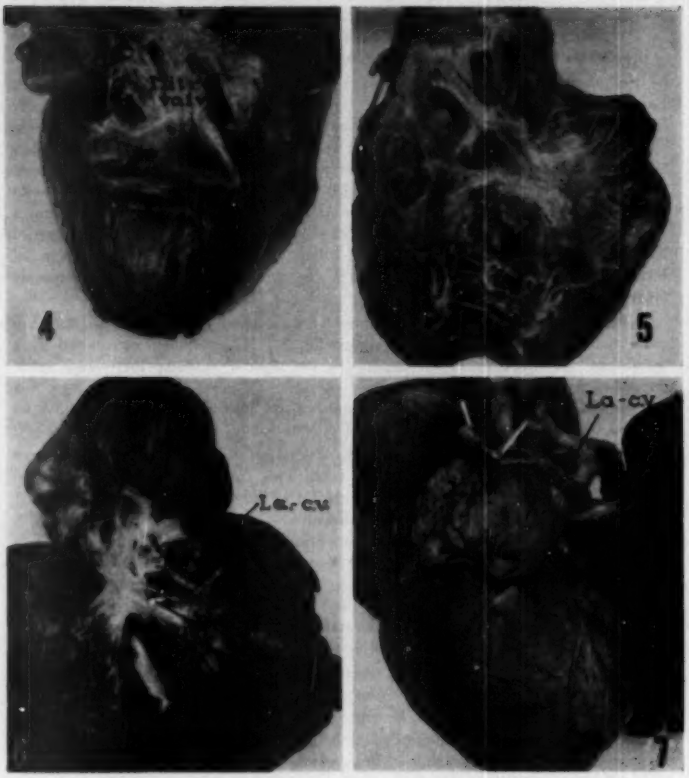


Fig. 4 (case 1).—Left atrium viewed from above; the anterior surface of the heart to left; posterior to right. At the expected location of the mitral valve (lower end of leader) there is neither opening nor valvular tissue. There is merely a depression. The atrial septum is an intact membrane.

Fig. 5 (case 1).—Interior of right atrium and ventricle. The limbus of the fossa ovalis is prominent. Right atrial and ventricular dilatation. Right ventricular hypertrophy. Nodular thickening of tricuspid leaflets.

Fig. 6 (case 1).—Lungs and dorsal wall of left atrium viewed from in front. The heart and the left pulmonary artery have been deflected upward. Medial to the left atrial entrance of the left superior pulmonary vein is the mouth of the levoatriocardinal vein (containing probe). As it passes upward this vein lies posterior to the left pulmonary artery. *La.-c. v.* indicates the levoatriocardinal vein.

Fig. 7 (case 1).—Left anterior view of thoracic organs. The levoatriocardinal vein (*La.-c. v.*) has ascended behind the left pulmonary artery and in its vertical course passes lateral to the ductus arteriosus. The levoatriocardinal vein joins the lower aspect of the origin of the left innominate vein (*L. I. V.*). Each probe lies in the course of a corresponding internal jugular vein. The branches of the aortic arch lie in the background.

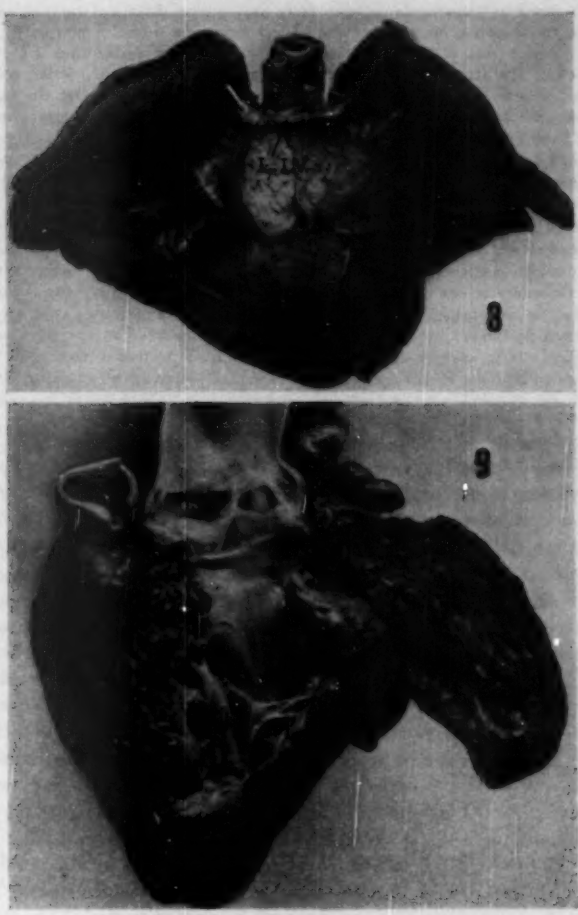


Fig. 8 (case 1).—The thoracic organs from in front. The left innominate vein (*L. I. V.*) courses normally to the right along the superior aspect of the thymus. From this view the right ventricle is seen to form the major portion of the anterior aspect of the ventricular part of the heart.

Fig. 9 (case 1).—Left ventricle. The membranous portion of the ventricular septum is intact. There are several defects of the muscular portion. The aorta arises in a normal position from the left ventricle. The photograph is somewhat enlarged.

The ventricular septum was of considerable interest. The membranous part was intact, while in the muscular portion there were several defects of varying size and shape, the largest being crescent shaped and measuring 6 by 2 mm. (fig. 9). The aortic orifice was 8 mm. in diameter. The pulmonary orifice was 1.2 cm. in diameter. The arch of the aorta, after giving off the innominate and left common carotid arteries in a normal manner, narrowed precipitously, so that the segment between the left common carotid artery and the left subclavian artery measured only 3 mm. in diameter. The isthmus of the aorta measured the same. The aorta widened as the descending portion was formed. The latter measured 7 mm. in diameter. The course of the descending aorta was normal. The coronary arteries arose from the aorta.

Microscopically, the myocardium was not remarkable. The endocardium of the left atrium was greatly thickened by fibrous tissue, predominantly elastic and to a lesser degree collagenous. Microscopic examination of the tricuspid valve revealed that the excrescences observed grossly were formed of loose cellular connective tissue. No infiltrating leukocytes were present. The appearance was like that described by Gross [1] as occurring in cardiac valves of infants. The latter author compared the appearance of the loose connective tissue to that of embryonic tissue and stated that the excrescences did not represent lesions of inflammatory nature. The lungs showed a marked degree of congestion, both grossly and microscopically.

COMMENT ON CASE 1

The first case will be discussed from the aspects of the unusual association of malformations and congenital mitral atresia, the routes of the circulation and the developmental basis of the anomalous vessel by which the left atrium communicated with the left innominate vein.

In the usual case of mitral atresia the arterialized blood from the lungs enters the left atrium by way of the pulmonary veins, as it did in the case here recorded. However, in the usual case the only outlet from the left atrium is a patent foramen ovale; thus all the arterialized blood reaches the right atrium. Here it mixes with venous blood entering the right atrium through the venae cavae and the coronary sinus. In the current case there was closure of the foramen ovale. Thus no direct channel, with the possible exception of small thebesian veins, was present between the atriums. The anomalous vessel which connected the left atrium with the left innominate vein therefore acquires some importance since, for practical purposes, it represented the only route of exit of blood of the left atrium. Through this channel blood ultimately entered the superior vena cava and the right atrium. Thus the case of mitral atresia here recorded is unusual in that the anatomic connection of the two atriums was an indirect one. Yet functionally it was like the usual case of the valvular malformation in that the arterialized and venous blood mixed in the right atrium. In this way the heart, in cases of mitral atresia, functions as a two-chambered heart.

1. Gross, P.: Arch. Path. **31**:163, 1941.

It is common in cases of mitral atresia for a common ventricle to be present and for the great arteries to be transposed.[2] In cases wherein there are two ventricles there is usually a defect in the membranous portion of the ventricular septum. In our case there was no transposition of the great vessels and the membranous portion of the ventricular septum had formed. The two ventricles communicated, however, through multiple defects of the muscular ventricular septum. Thus all the blood returning to the heart, both from the periphery and from the lungs, flowed from the right atrium through the tricuspid orifice into the enlarged right ventricle. From this chamber some of the blood flowed in a normal direction into the pulmonary trunk. The rest of the blood in the right ventricle passed through the several perforations in the muscular portion of the ventricular septum to the left ventricle. From this chamber blood flowed to the aorta and to its branches.

The ductus arteriosus, although admitting a probe, was thick and narrower than the average ductus at birth. This leads us to the assumption that the ductus arteriosus was in the process of orderly closure. In this case this was of no functional consequence. If, however, the ventricular septum had been intact, the only means left by which the aorta could have received blood would have been the ductus arteriosus. Therefore, closure would have interfered with blood reaching the aorta.

The developmental basis of congenital mitral atresia is not clearly understood. In an earlier communication from the Mayo Clinic the hypotheses were cited.[2b] In general, it is felt by some that the basis lies in some fault in fundamental growth. Others have expressed the opinion that mitral atresia results from endocarditis occurring during fetal life. The association of premature closure of the foramen ovale of the type here described deserves some comment in a consideration of the basis of the mitral atresia since the two conditions are conceivably related. Before this is mentioned, it is pertinent to make some comments on the anatomic state of the atrial septum in our case and to give the anatomic explanation for the foramen ovale being prematurely closed.

Premature closure of the foramen ovale is a rare condition which usually occurs without associated cardiac malformations.[3] In some of the cases it appears to result from overgrowth of the septum secundum of the atrial septal complex.[4] It will be recalled that in the development of the definitive atrial septum two septums, the septum primum and the septum secundum, are involved. The septum secundum, which appears after the septum primum has become manifest, lies to the right

2. (*a*) Scriba, K.: Zentralbl. f. allg. Path. u. path. Anat. **67**:353, 1937. (*b*) Edwards, J. E., and Rogers, H. M.: Internat. A. M. Museums Bull. **27**:62, 1947.

3. (*a*) Lehman, E.: Am. J. Dis. Child. **33**:585, 1927. (*b*) Benner, M. C.: Am. Heart J. **17**:437, 1939.

4. Patten, B. M.: Am. J. Path. **14**:135, 1938.

of the septum primum. In the septum secundum it is normal that an opening, the foramen ovale, be present. The septum secundum may be compared to a doughnut wherein the substance of the doughnut is the substance of the septum. The hole in the doughnut is the foramen ovale. If the tissue of the septum secundum grows beyond its bounds, the foramen ovale may become unduly narrow or be obliterated completely. Should this happen there would be premature closure of the foramen ovale. When inspected from the right, the atrial septum would fail to show the normal configuration with a foramen ovale. The abnormal process in the formation of the atrial septum so far outlined is not, however, applicable to our case, since there was a clearly evident foramen ovale. There must be other causes for premature closure of the foramen ovale. Normally in the embryo, as the septum primum is about to complete its growth and to fuse with the atrioventricular endocardial cushions, a secondary opening develops in this septum. This opening is the interatrial foramen secundum. Thus there is provided a route for blood to pass from the right atrium into the left throughout fetal life.[4] The route is for blood to pass progressively from right to left through the foramen ovale, between the septum secundum and the septum primum and then into the left atrium through the interatrial foramen secundum in the septum primum. The route is oblique, the opening in the septum primum being forward of, and superior to, the foramen ovale in the septum secundum. The tissue in the septum primum behind the interatrial foramen secundum is the valve of the foramen ovale. It is evident that there would be no channel between the two atria (1) if the interatrial foramen secundum failed to form or (2) if the valve of the foramen ovale fused with the septum secundum during fetal life, as it does in many normal infants some weeks or months after birth.

From an examination of our case it is our opinion that the foramen ovale was closed because of one of the two conditions suggested, but it is not possible to state which one. If the valve of the foramen ovale fused with the septum secundum after there had been an opening in the septum primum, an inflammatory basis for the closure would have to be considered.

To return to the question of the basis of the mitral atresia, it is tempting to consider that this malformation, as well as the premature closure of the foramen ovale, could have been due to one process, namely, endocarditis. The anatomic proximity of the expected location of the mitral valve and the atrial septum makes an inflammatory cause for both the mitral atresia and the premature closure of the foramen ovale an interesting point for speculation. The evidence would have to be more concrete, however, for one to be able to draw conclusions as to the basis of the valvular and septal malformations.

The vascular malformation characterized by a channel connecting the left atrium with the left innominate vein is rare. As far as we can ascertain there are but 2 other established cases with comparable connections. These are the case of McIntosh [5] and the case of Harris, Gray and Whitney.[6] In the case of Bellet and Gouley [7] a similar connection is suggested. The case of McIntosh is particularly interesting and pertinent to mention since in it there were mitral atresia and a closed foramen ovale as in our case. The anomalous vein in the case of McIntosh connected the left atrium with the superior vena cava. In the case of Harris and associates there was complete transposition of the great vessels. An anomalous vein connected the left atrium with the right internal jugular vein. There was also a persistent left superior vena cava which connected, in the usual fashion, with the coronary sinus.

In the case of Bellet and Gouley a similar vascular connection is suggested. The patient of the latter authors was an infant who died at the age of 12 hours. The heart showed atresia of the aortic orifice, a closed ventricular septum and premature closure of the foramen ovale. The mouths of the pulmonary veins were identified in the posterior wall of the left atrium. More anteriorly there was the mouth of an additional vessel, but since the necropsy was restricted to an examination of the heart, the specimen did not contain the termination of the additional vessel. Bellet and Gouley suggested that this vessel might have had connections comparable with those of the anomalous vessel in the case of McIntosh, that is, between the left atrium and the superior vena cava. As indicated, the nature of the specimen did not allow a conclusive opinion on this matter.

At first glance it seems difficult to explain on a developmental basis the cause of the anomalous connection between the left atrium and the left innominate vein in our case or the connections described by McIntosh and by Harris and associates. These anomalous connections seem intimately concerned with the development of the pulmonary veins. A consideration of the origin of the normal pulmonary veins seems pertinent as background for the understanding of the anomalous connections under consideration.

According to Patten,[8] the pulmonary veins develop by differentiation of a capillary plexus which surrounds the primitive foregut, trachea and lung buds. By a process of coalescence of the elements of the capillary plexus a single vein leaves each pulmonary lobe. These individual veins

5. McIntosh, C. A.: Am. Heart J. **1**:735, 1926.

6. Harris, H. A.; Gray, S. H., and Whitney, C.: Anat. Rec. **36**:31, 1927.

7. Bellet, S., and Gouley, B. A.: Am. J. M. Sc. **183**:458, 1932.

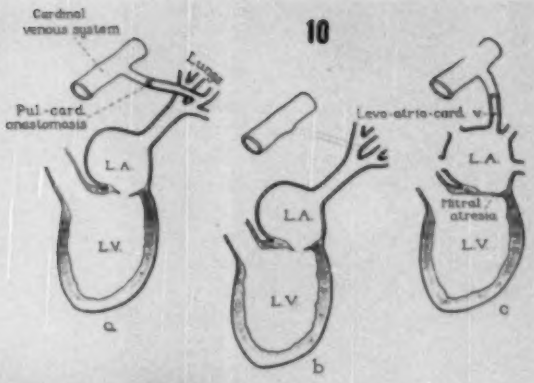
8. Patten, B. M.: Human Embryology, Philadelphia, The Blakiston Company, 1946, pp. 644 and 674-675.

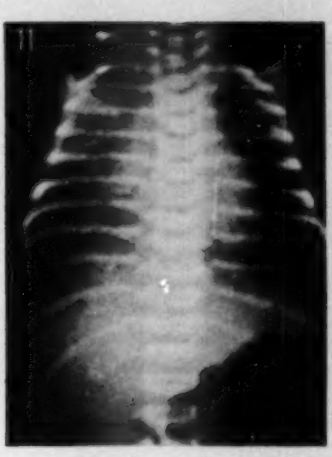
converge to form a common pulmonary vein (fig. 10 *a*). The latter joins the left atrium. By a process of differential growth the common pulmonary vein and its tributaries become incorporated into the dorsal wall of the left atrium. This results in the usual adult condition wherein four individual pulmonary veins enter the left atrium independently (fig. 10 *b*).

Let us return to a consideration of the early stages of the development of the pulmonary veins, to the stage where the precursors of the pulmonary veins are represented as a capillary plexus. This plexus has connections with capillaries of the developing esophagus and with the cardinal veins. From the latter, among other structures, will develop the adult innominate and jugular veins, the superior vena cava and the coronary sinus. Usually the connections of the pulmonary venous primordia on one hand with the esophageal capillaries and the cardinal venous system on the other hand are lost. If connections with the cardinal veins are retained and the connection with the main pulmonary vein is lost, the pulmonary vein concerned will not drain into the left atrium but into one of the derivatives of the cardinal system of veins. This is the basis for certain cases with anomalous drainage of pulmonary veins. No such condition was present in our first case, however.

To explain the anomalous venous connection between the left atrium and the left innominate vein in our case, the following assumptions seem justified: The embryonic capillary plexus about the left lung bud retained its connection both with the main pulmonary venous trunk and at the same time with the cardinal venous system (fig. 10 *a* and *c*). With differential growth, as is normal, the individual pulmonary veins joined the left atrium independently. Likewise by the process of differential growth the retained connection between the venous system of the left lung and the cardinal venous system became incorporated into the wall of the left atrium. This resulted in the gross anatomic situation of a direct communication between the left atrium and the cardinal venous system. In our first case that portion of the embryonic cardinal venous system connected with the pulmonary venous system became the left innominate vein. In the case of McIntosh the portion involved became the superior vena cava, and in the case of Harris and associates the right internal jugular vein was developed from that portion of the cardinal venous system which maintained its connection with a pulmonary vein.

It seems pertinent that a name be given to such anomalous vascular connections as are being discussed. Unless a standard name is adopted, classification becomes difficult and reported cases are lost in the maze of the literature. Harris and associates termed the anomalous connection in their case a "jugulo-pulmonary vein." This seems correct, but since the anomalous connection seems to be able to involve several derivatives





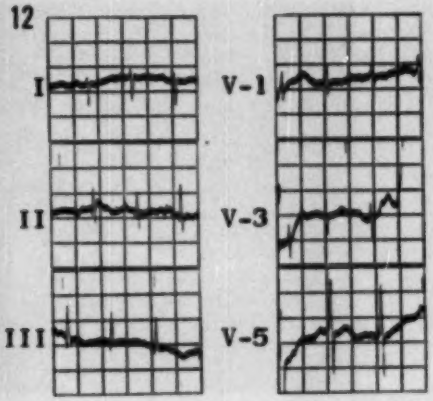


Fig. 10 (case 1).—Diagrammatic representation of stages in the development of the pulmonary veins and of the concept as to the developmental basis for the connection found between the left atrium and the left innominate vein in our first case. *L. A.* is the left atrium; *L. V.*, the left ventricle.

(*a*) This is representative of an early embryonal stage. From each pulmonary lobe a vein leaves. The veins converge to form a common pulmonary vein which in turn connects with the left atrium. In early periods there are connections between the pulmonary venous primordia and the cardinal venous system. One such connection is portrayed.

(*b*) At a stage later than that shown in *a* the connections between the developing pulmonary veins and the cardinal venous system are lost. The common pulmonary vein is gradually incorporated into the dorsal wall of the left atrium as a result of differential growth.

(*c*) The final stage in the development of the pulmonary veins. By a process of differential growth the common pulmonary vein and its tributaries from the individual lobes have become incorporated into the wall of the left atrium. The pulmonary veins now enter the left atrium independently. The connection between the pulmonary venous primordia and the cardinal system of veins, as shown in *a*, has been maintained and so carried into the wall of the left atrium along with the pulmonary vein to which it has been connected. In this way the levoatriocardinal vein seems to be developed.

Fig. 11 (case 2).—The roentgenogram of the thorax.

Fig. 12 (case 2).—The electrocardiogram.

of the cardinal system of veins, a more inclusive name would seem justified. In discussing the matter with Dr. John R. Miner, of the Section on Publications of the Mayo Clinic, it was decided that an anomalous connection of the category being discussed could conveniently be named a "levoatrio-cardinal vein." This name is recommended by us.

Mention should be made of the venous abnormality in case 3 of Potter.[9] The case was that of a newborn infant who died at the age of 32 hours. In addition to diffuse angiectasis of the cerebral meninges there were intrathoracic vascular abnormalities. The left innominate vein, instead of uniting with the right innominate vein to form the superior vena cava, entered the lateral wall of the left atrium anterior to the pulmonary veins. From the published photograph of the specimen it appears that the vein also passed anterior to the left pulmonary artery. Thus, the left pulmonary artery was interposed between the anomalous vein and the left pulmonary veins. In our case the connections of the left pulmonary veins and of the levoatriocardinal vein with the left atrium were all behind the left pulmonary artery. From the course and position of the anomalous vessel in Potter's case one is led to the interpretation that it is a persistent left superior vena cava with an abnormal, secondary communication with the left atrium, rather than a vessel such as ours which in its position seems to be intimately related to the formation of the pulmonary veins.

CASE 2

A 6 day old boy was admitted to the hospital, April 21, 1949, because of dyspnea and cyanosis since birth. The birth was not unusual and followed a normal pregnancy. Immediately after delivery of the infant, rapid respirations and cyanosis were noted. The cyanosis was accentuated by crying. With constant administration of oxygen the skin remained pink. Examination revealed a dyspneic infant whose color was good in the oxygen tent. The heart was not enlarged, and there were no murmurs.

Laboratory studies revealed albuminuria, grade 1. There was 17.2 Gm. of hemoglobin in 100 cc. of blood. Erythrocytes numbered 4,440,000 and leukocytes 7,600 per cubic millimeter of blood. The results of roentgenologic examination of the heart and lungs were negative (fig. 11). The electrocardiogram is reproduced in figure 12.

In spite of constant administration of oxygen, the infant became more dyspneic and tachypneic. He died three days after being admitted to the hospital. The clinical diagnosis was indeterminate, although lobular atelectasis was suspected.

Pathologic Features.—Externally the heart showed some enlargement of the right atrium. The great vessels arising from the ventricular cavities were properly interrelated. Examination of the interior of the heart revealed that the venous connections of the right atrium were normal. The tricuspid valve was normal. The right ventricular cavity was larger than the left, and its wall was somewhat thicker than that of the left. The pulmonary trunk arose from the right ventricle. The orifice was guarded by three normal semilunar cusps. The ductus arteriosus

9. Potter, E. L.: Arch. Path. **46**:87, 1948.

Fig. 13 (case 2).—Left atrium and ventricle. The foramen ovale is patent. This is the only channel through which the left atrium receives blood. The thebesian veins in the atrial septum are wide.

Fig. 14 (case 2).—The thoracic organs viewed from in front. The heart has been deflected to the right. The common pulmonary vein (*C. P. V.*) fails to join any chamber of the heart. The left atrium is small.

Fig. 15 (case 2).—The thoracic organs viewed from behind. Leaving each lung are two veins which converge to form a common pulmonary vein (*C. P. V.*).

Fig. 16 (case 2).—The diaphragm and part of the liver from above. The common pulmonary vein (*C. P. V.*) leaves the thorax by passing through the diaphragm in company with and in front of the esophagus.

Fig. 17 (case 2).—The upper abdominal organs from in front. The lower margin of the liver has been turned upward to expose the hilus. The common pulmonary vein has been pulled through the diaphragm. This vein terminates in the patent but narrow ductus venosus. The portal veins are normal. *C. P. V.* is the common pulmonary vein; *Ductus v.*, the ductus venosus; *L. P. V.*, the left portal vein; *R. P. V.*, the right portal vein; *P. V.*, the portal vein; *G. B.*, the gallbladder.

Fig. 18 (case 2).—The hilus of the liver. The left lobe of the liver has been deflected well to the right. The ductus venosus (containing probe) runs in a normal fashion from the left portal vein (*L. P. V.*) to the left hepatic vein. The ductus venosus receives the common pulmonary vein (*C. P. V.*). *R. P. V.* is the right portal vein; *P. V.*, the portal vein.

was thick walled. It would admit only a fine probe. From its appearance it seemed to be undergoing normal closure. Examination of the left atrium revealed that the cavity was smaller than the right atrium and considerably smaller than normal. No veins except the thebesian veins entered the left atrium. There was an atrial septal defect in the form of a patent foramen ovale measuring 1.0 cm. in diameter (fig. 13). The thebesian veins in the atrial septum were wide. The ventricular septum was intact.

The pulmonary venous drainage was anomalous. Leaving the lungs were two veins on each side, an upper and a lower. These converged anterior to the esophagus to form a common pulmonary venous trunk (figs. 14 and 15). None of the pulmonary veins perforated the pericardium or joined the left atrium (fig. 14). The common pulmonary venous trunk descended anterior to the esophagus and passed through the diaphragm in company with the esophagus (fig. 16) to enter the abdominal cavity. Just beneath the diaphragm the common pulmonary vein took an acute turn to the right, coursed beneath the left lobe of the liver and ended by joining the ductus venosus (figs. 17 and 18). The portal vein of the liver was normally formed and branched normally into right and left branches. The umbilical vein showed signs of becoming obliterated. The ductus venosus was patent but narrow. Its lumen measured approximately 2 mm. in diameter.

The lungs were firm, subcrepitant and reddish purple. When cut they were moist and exuded a small amount of frothy, bloody fluid. Microscopically the lungs showed a considerable degree of congestion. Many of the capillaries contained 5 or 6 erythrocytes at the level of one cross section. The remainder of the organs, including the heart, showed no unusual microscopic features.

COMMENT ON CASE 2

Reports on anomalous drainage of the pulmonary veins are uncommon. In 1942 Brody [10] reported on 106 cases in which necropsy was performed and in which the pulmonary veins drained completely or partially into the right atrium or into one of its tributaries. The report included 4 cases which he had observed personally and 102 which had been reported by others. In this collection of cases there were 38 in which the anomalous drainage was complete and 68 in which it was partial. Partial anomalous drainage of the pulmonary venous system causes a functional disturbance which is closely allied to the alterations in cases of atrial septal defect; that is, the characteristics of an arteriovenous shunt are present. An anomaly in which the pulmonary veins drain completely into the right atrium or into one of its tributaries functions differently. In many ways it functions as though a two-chambered heart were present. In 1947 Young [11] collected data on certain reported cases of anomalous pulmonary venous drainage which Brody had not mentioned. These included several reported since the time of Brody's paper. Young's collection brought the total number of reported instances of anomalous drainage of the pulmonary veins up to 127. Young added a case of his own in his report. If to this figure

10. Brody, H.: Arch. Path. **33**:221, 1942.

11. Young, M. O.: Arch. Path. **44**:169, 1947.

are added the case of Hu,[12] reported in 1929, and mentioned by Mehn and Hirsch [13] but not by Brody or Young, and the cases of Mehn and Hirsch, of Mykschowszky,[14] of Graham [15] and of Kernan,[16] the total of cases of anomalous pulmonary venous drainage in which necropsy was performed would be brought up to 132.

Doubtless there are many other cases studied pathologically which have not come to light because of the fact that the titles of papers reporting these anomalies do not suggest the presence of anomalous pulmonary veins. The case observed by Feldman and Chalmers [17] is a case in point. Their patient had complete transposition of the great vessels. In addition, all of the pulmonary veins drained into the right atrium. This case is not mentioned in existing reviews of pulmonary venous anomalies. Moreover, it is our opinion that unless at necropsy dissection including the thoracic organs, at least, in one block is done the chance of a pathologist's noting an anomalous condition of the pulmonary veins is practically negligible. Therefore, with the technic of removing the organs separately being practiced at necropsies by so many pathologists, it seems correct to judge that a number of these malformations are never discovered. Cases of anomalous pulmonary veins discovered at operation [18] or on clinical investigation [19] have also been reported.

As stated, the anomalous draining of the pulmonary veins into the right atrium or into one of its venous tributaries may be either partial or complete. In cases of partial anomalous drainage some of the pulmonary veins enter the left atrium normally. The anomalous veins carry arterialized blood to the right side of the heart. The functional effect is that of an arteriovenous shunt, similar in many ways to the effect of an atrial septal defect. In this discussion no further emphasis will be placed on partial anomalous pulmonary venous drainage.

In cases of complete anomalous pulmonary venous drainage all the arterialized blood which leaves the lungs reaches the right atrium, wherein it mixes with venous blood. This mixture is then distributed partly to the pulmonary arterial system and partly through the foramen ovale to the left side of the heart and the greater arterial circulation.

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12. Hu, C. H.: Am. J. Path. **5**:389, 1929.
13. Mehn, W. H., and Hirsch, F. E.: Am. J. Path. **23**:125, 1947.
14. Mykschowszky, G.: Klin. med. **3**:263, 1948.
15. Graham, P. M.: M. J. Australia **2**:545, 1944.
16. Kernan, J. D.: Ann. Otol., Rhin. & Laryng. **53**:818, 1944.
17. Feldman, W. H., and Chalmers, A.: Brit. J. Child. Dis. **30**:27, 1933.
18. Brantigan, O. C.: Surg., Gynec. & Obst. **84**:653, 1947.
19. (*a*) Johnson, A. L., and McRae, D. L.: Pediatrics **2**:643, 1948. (*b*) Dotter, C. T.; Hardisty, N. M., and Stienberg, I.: Am. J. M. Sc. **218**:31, 1949.

Complete anomalous drainage therefore functions, in regard to intracardiac mixing of blood, as a two-chambered heart. It is quite different functionally from partial anomalous pulmonary venous drainage.

Complete anomalous drainage of the pulmonary veins may be associated with congenital cardiac disease, or the heart may be normal, showing only the expected disproportion between the size of the right side of the heart and that of the left. The right side is usually enlarged and the left side is smaller than normal. When the pulmonary veins drain completely into the right atrium or into one of its tributaries, the foramen ovale is patent, the patency usually being in the form of a valvular patency as is seen in the fetus. This should be considered not as a malformation but as a natural response to the phenomenon of blood continuing to flow after birth from the right atrium into the left as before birth. In such cases the left atrium has no source of blood other than the channel of the foramen ovale.

The anomalous pulmonary venous connection or connections may be made directly into the right atrium or into one of its tributary veins. Veins receiving the anomalous pulmonary veins in complete anomalous drainage are usually the structures derived from the cardinal system of veins, as, for example, the superior vena cava, the coronary sinus or the left innominate vein. Much less commonly the anomalous drainage is into the portal vein or into the ductus venosus. Of the 38 cases of complete anomalous drainage of the pulmonary veins which Brody discussed, only 5 showed these veins draining into the portal vein and 1 into the ductus venosus. The latter was the case of Ghon.[20] Since the report of Brody the following reports of pulmonary veins draining below the diaphragm into structures as indicated have been reported: Young, portal vein; Mehn and Hirsch, ductus venosus, and Mykschowszky, portal vein. In the case of Hu, to which reference has already been made, the pulmonary veins drained completely into the portal vein. Our case 2, in which the pulmonary venous system drained wholly into the ductus venosus, should be added to this group.

It is evident from the foregoing that as far as we are aware there are only 3 cases, including ours, of the anomaly of pulmonary veins draining into the ductus venosus. These are the cases of Ghon, Mehn and Hirsch and ourselves. The 3 cases are very similar, pathologically and clinically. In each the subject was a cyanotic newborn infant dying within three weeks after birth. Ghon's subject died at the age of 15 days, Mehn and Hirsch's at 12 days and ours at 9 days. In each the right side of the heart was larger than normal and the left side was significantly smaller than normal. A patent foramen ovale was present in each. No other cardiac changes of significance were present in Ghon's and our cases. The specimen studied by Mehn and Hirsch

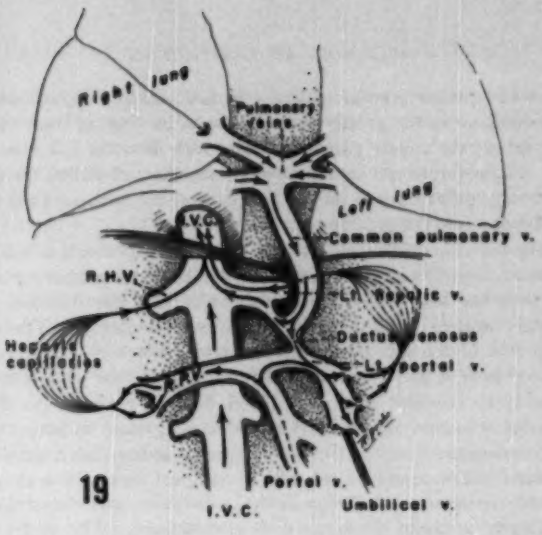
20. Ghon, A.: Beitr. z. path. Anat. u. z. allg. Path. 62:175, 1916.

showed a muscular ventricular septal defect and coarctation of the aorta, the latter being located between the origin of the left subclavian artery and the patent ductus arteriosus.

The circulatory routes in our case are shown in figures 19 and 20. Blood leaving the lungs flowed to the ductus venosus. From this vessel some of the blood probably flowed to the left hepatic vein and so to the inferior vena cava. The rest probably flowed into the portal system by way of the left portal vein. The latter blood would have had to traverse the hepatic sinusoids before being delivered to the inferior vena cava. This factor must have added to the resistance to pulmonary venous blood flow by virtue of resistance at the level of the hepatic sinusoids. This, coupled with the narrow state of the ductus venosus, must have been a considerable barrier to the emptying of the pulmonary veins.

This was evidently reflected in the existing pulmonary congestion. The barrier to venous flow seemed eventually to have caused increased work to fall on the right ventricle. If the load exceeded the capabilities of the right ventricle, which it may well have done, this would have caused incomplete systolic emptying of the right ventricle. This, in turn, would have raised the right ventricular diastolic pressure. The latter, in turn, would have constituted a barrier to the emptying of the right atrium through the tricuspid orifice. This would tend to make more blood pass through the foramen ovale into the left atrium. The result of these alterations would be a greater systemic blood flow coupled with decreased pulmonary blood flow. Since the arterialized blood coming into the heart was blood which had passed through the anomalous channel and into the inferior vena cava, the blood entering the right atrium would contain less arterialized blood than under conditions of no increased resistance to pulmonary venous flow. Cyanosis, which could originally have been present merely on the basis of the left side of the heart receiving a mixture of venous and arterialized blood, would increase in intensity by virtue of diminished pulmonary blood flow. Similar circulatory disturbances seem to have existed in the cases of Ghon and of Mehn and Hirsch.

It seems correct to state that the functional disturbances caused by the anomalous pulmonary venous system that drained wholly into the ductus venosus in our second case are comparable to those of the tetralogy of Fallot. This is so in spite of the profoundly different anatomic arrangements. Each condition has pulmonary stenosis and a means of venous blood entering the greater circulation. In the tetralogy the pulmonary stenosis is at, or in the vicinity of, the pulmonary orifice; in our second case the barrier to pulmonary blood flow was beyond the lungs, at the narrow ductus venosus and the sinusoidal bed of the liver. In the tetralogy the biventricular origin of the aorta allows venous blood



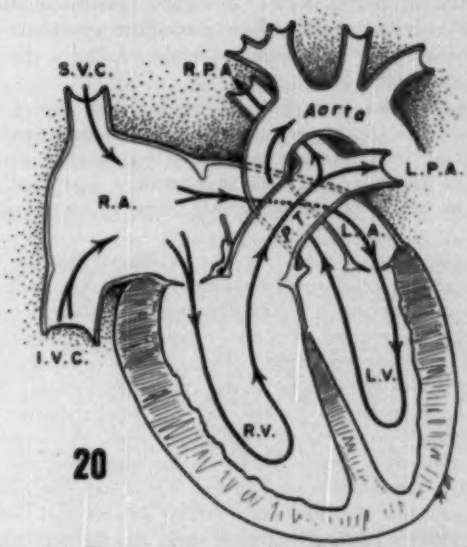


Fig. 19 (case 2).—The anomalous connections of the pulmonary veins. *I. V. C.* is the inferior vena cava; *R. H. V.*, the right hepatic vein; *R. P. V.*, the right portal vein. (From Edwards, J. E.: Functional Pathology of Certain Cardiovascular Malformations Which May Be Treated Surgically, to be published.)

Fig. 20 (case 2).—The intracardiac circulation. *I. V. C.* is the inferior vena cava; *L. A.*, the left atrium; *L. P. A.*, the left pulmonary artery; *L. V.*, the left ventricle; *P. T.*, the pulmonary trunk; *R. A.*, the right atrium; *R. P. A.*, the right pulmonary artery; *R. V.*, the right ventricle; *S. V. C.*, the superior vena cava. (From Edwards, J. E.: Functional Pathology of Certain Cardiovascular Malformations Which May Be Treated Surgically, to be published.)

to enter the greater arterial system. In our second case venous blood gained entrance to the greater arterial system by flowing from the right atrium across the widely patent foramen ovale into the left side of the heart. Moreover, in our case 2, as in the tetralogy of Fallot, the greater the barrier to pulmonary blood flow, the greater the volume of the venous blood flowing into the greater circulation.

Were the clinical diagnosis of the existing malformation made and were there surgical means of connecting the common pulmonary venous trunk with the left atrium, it is conceivable that the function of the circulatory system could have been returned to normal. The underdevelopment of the left atrium and ventricle, however, could conceivably have been a barrier to normal function. At the same time it is reasonable to consider that with blood flowing normally to the left atrium the left side of the heart would have grown in proportion to the demands placed on it. In view of the possibility that normal cardiovascular function could be restored by surgical means in such a case, the problem remains a challenge to the pediatrician and the cardiologist as to diagnosis and to the surgeon as to treatment. The lesson in the diagnosis of our case lies in the fact that the physical and roentgenologic findings were negative in the face of evident cyanosis of the newborn. Though this may be a cardinal sign of complete anomalous pulmonary venous drainage, other malformations might produce a clinical picture of similar character.

The developmental basis of the anomalous connection of the pulmonary veins and the ductus venosus seems to include a combination of factors. These are a maintenance of embryonic connections ordinarily temporary in nature, evolution of an anomalous vein from these connections and failure of the pulmonary veins to unite with the left atrium.

In the comment on case 1 it was explained that the pulmonary venous anlages are represented by a capillary plexus which surrounds the foregut of the embryo. With the growth of the embryo the pulmonary veins are evolved from this plexus, and with further growth they connect with the left atrium. During the early stages of development the primitive connections of the pulmonary venous anlages with the cardinal venous system are lost, and in a similar manner the developing pulmonary veins sever connection with the vessels of the developing alimentary canal. In the region concerned, the esophagus represents the alimentary tract. In our second case the anatomic evidence indicates that the pulmonary venous anlages had normally lost connection with the cardinal system of veins, and the developing pulmonary veins did not communicate with the left atrium. From the gross connections existing at necropsy one must assume that that part of the capillary plexus which was to give rise to the pulmonary veins

had maintained anatomic continuity with those capillaries which were destined to represent the blood supply of the esophagus. With these connections representing the only outlet for blood coming from the lungs, there developed from the periesophageal capillary plexus a gross venous channel. The latter appeared grossly as the common pulmonary vein. The lower portion of the common pulmonary vein is interpreted as being derived from the primitive vascular supply of the stomach, which has vascular continuity with the esophagus. The venous supply of the gastrointestinal tract and the umbilical venous circulation must be reviewed to serve as a basis for the understanding of the connection of the lower end of the common pulmonary vein with the ductus venosus. The normal developmental story to follow is taken from Patten.[8]

The primitive gastrointestinal tract shares its venous supply with the yolk sac, the venous supply being the omphalomesenteric veins. These pass craniad and terminate in the sinus venosus of the embryonic heart. As the liver develops, it comes into close contact with the omphalomesenteric veins, and the hepatic cords break up these veins into a network of small channels which are intimately related with the developing hepatic cords. Later the caudal portions of the omphalomesenteric veins become differentiated into the portal vein and its main tributaries.

The original umbilical venous connections are, like the omphalomesenteric, directly with the sinus venosus of the heart, and like the latter vessels the umbilical veins become interrupted into a capillary network by the developing liver. Later a channel is molded from intrahepatic capillaries whereby the blood from the umbilical vein largely avoids the hepatic capillaries and is carried directly to the left hepatic vein and so to the heart. The channel which develops between the umbilical and the left hepatic vein is known as the ductus venosus. It is evident that at one stage all the venous drainage of the gastrointestinal tract and the placenta empties into the hepatic capillaries. While normally the ductus venosus is so connected and situated as to carry placental blood, the ductus venosus develops from capillaries that at one time contained intestinal blood as well. It is therefore possible for a vein carrying blood from the gastrointestinal tract to maintain a connection with those capillaries destined to become the ductus venosus and so ultimately to result in the connection of a gross gastrointestinal vein with the ductus venosus.

In our second case the lower end of the common pulmonary vein seems to be interpreted correctly as a gastrointestinal vein from the point of view of its origin. As such its connection with the ductus venosus becomes an understandable phenomenon.

VALUE OF GASTRIC BIOPSY SPECIMENS OBTAINED THROUGH THE FLEXIBLE OPERATING GASTROSCOPE

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THE FIRST successful biopsy of the stomach made by means of the flexible operating gastroscope [1] was made on March 4, 1948.[2] Since then 63 specimens have been taken for biopsies with no complications and no accidents. The flexible operating gastroscope originally designed was of rather large caliber for general use, being 17.5 mm. at its largest diameter. In a few instances it was impossible to pass it, owing to its large size. The new operating gastroscope is 15 mm. at its largest diameter; it is more flexible than the first model and easy to pass.

As previously reported,[3] biopsy has proved useful in the diagnosis of lymphoma, carcinoma and gastritis. It is well known that heretofore it has been impossible to differentiate lymphoma from severe gastritis. A gastroscopic biopsy that demonstrates lymphoma offers positive proof of the disease; a gastroscopic biopsy that does not demonstrate lymphoma may exclude lymphoma with reasonable certainty, because this growth is generally regarded as a diffuse process. In differentiating lymphoma from gastritis a gastroscopic biopsy is very important, lymphoma of the stomach being a surgical problem and gastritis a medical problem.

The diagnosis of carcinoma of the stomach may be easy or difficult. In a difficult case a positive gastroscopic biopsy establishes the diagnosis beyond any question. A biopsy that does not reveal the presence of carcinoma, on the other hand, does not exclude the lesion, because carcinoma may be a localized process and the specimen may have been taken from a noninvolved area near a carcinoma.

The 63 specimens so far obtained have been reported by the pathologist as showing normal stomach (18), chronic gastritis (24), acute

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From the Massachusetts General Hospital.

Read before the Section on Gastro-Enterology and Proctology, at the Ninety-Eighth Annual Session of the American Medical Association, Atlantic City, June 10, 1949.

1. This instrument is manufactured by the American Cystoscope Makers, Inc., New York.

2. Benedict, E. B.: Gastroenterology **11**:281-283, 1948.

3. Benedict, E. B.: (*a*) Gastroenterology **14**:275-279, 1950. (*b*) New England J. Med. **241**:152-154, 1949.

and chronic gastritis (10), inadequate specimen (6), carcinoma (3) and lymphoma (2). The cancers have been discussed in detail in another report.[28]

GASTRITIS

Although the gastroscopic diagnosis of gastritis based on the gross appearance of the stomach as seen through the flexible gastroscope has become widely accepted, there is no doubt that a microscopic report is of assistance in putting the diagnosis of gastritis on a positive histologic basis. The study of biopsy specimens is also of value in correlating clinical, radiologic, gastroscopic and pathologic observations in gastritis. So far efforts in this direction have been based almost entirely on specimens resected for peptic ulcer or carcinoma.

Gastroscopic biopsy may (1) substantiate the gastroscopist's gross impression of gastritis, (2) demonstrate the presence of gastritis in a stomach which looks endoscopically normal or (3) fail to reveal pathologic evidence of gastritis in a stomach which appears grossly abnormal to the gastroscopist. The cases reported here illustrate these points.

Case 1.—H. R., a 70 year old man, was referred for gastroscopy because of repeated gastrointestinal bleeding. The past history revealed that three years prior to admission subtotal gastrectomy had been performed for bleeding duodenal ulcer. On several occasions massive bleeding occurred, requiring numerous transfusions. Roentgen examination of the gastric remnant revealed no involvement. Gastroscopy showed that throughout the gastric remnant the mucosa was edematous and hemorrhagic, with numerous actively bleeding erosions and linear areas of hemorrhage. There was some bright red blood free in the stomach. A satisfactory biopsy specimen was obtained from the mucosa on the greater curvature of the gastric remnant. The pathologist reported that the condition was acute and chronic gastritis.

Comment: In this case the gross appearance of the stomach was definitely that of acute gastritis with erosions and hemorrhages. The pathologist's report substantiated the gastroscopist's diagnosis.

Case 2.—W. J. F., a 42 year old man, entered the hospital with a history of epigastric distress of seventeen years' duration. About seventeen years prior to this he was operated on at another hospital for perforated ulcer. Fifteen years prior to admission a roentgenogram demonstrated a deformed duodenal cap. At that time gastroscopy demonstrated hypertrophic gastritis. One month prior to the present admission roentgenologic study revealed an active duodenal ulcer and probable gastritis. There were gross irregularity of the mucosal markings and a suggestion of rigidity high on the lesser curvature. The picture suggested the possibility of lymphoma. Two weeks later gastroscopy demonstrated intense reddening and edema of the mucosa throughout the body of the stomach with thin, tortuous, segmented folds which did not respond to inflation of air. The findings were those of very prominent acute superficial and chronic hypertrophic gastritis. Lymphoma was considered a definite possibility. The operating gastroscope was not available, and consequently a specimen could not be obtained for biopsy. About two weeks later gastroscopy was done with the operating

gastroscope, which revealed essentially the appearance previously described. A satisfactory specimen was obtained for biopsy, which was reported to show moderately severe acute and chronic gastritis with cystic dilatation.

Comment: In this case the gross appearance of acute and chronic gastritis was confirmed by the pathologist's report. Lymphoma, which is usually a diffuse process, was excluded with reasonable certainty by means of gastroscopic biopsy.

Case 3.—C. E. S., a 46 year old automobile worker, entered the emergency ward with a history of severe hematemesis of twenty-four hours' duration. There had been a heavy intake of alcoholic beverages for at least ten years, the average consumption being 1 pint (473 cc.) per day. The liver was enlarged, and a biopsy of the organ was reported as showing a condition consistent with early alcoholic cirrhosis. However, no esophageal varices could be found by either roentgenogram or esophagoscope. Roentologic examination of the stomach and duodenum gave negative results. Fifteen days after the hematemesis, gastroscopy demonstrated a normal-appearing stomach, but the gastroscopic biopsy specimen revealed slight acute and chronic gastritis.

Comment: This case illustrates the fact that the stomach may appear normal both in the roentgenogram and at gastroscopic examination and yet there may be some degree of acute and chronic gastritis, as demonstrated by pathologic examination. In the absence of esophageal varices or other demonstrable lesions it is probable that the patient's hematemesis came from the gastritis. That gastritis was present was proved only by gastroscopic biopsy.

Case 4.—A. M., a 55 year old physician, was referred for gastroscopy to differentiate severe gastritis from possible tumor. The present illness began four months prior to this when the patient began to have gnawing epigastric pain, relieved by milk or an aluminum hydroxide gel. The radiologist reported that the prominence of the mucosal pattern was considerably increased. The prominence of the rugae was particularly impressive in the middle third. The duodenal bulb was irritable, but no ulcer fleck was visible. The distortion of the mucosal pattern was more marked than it had been when the patient was examined four months prior to this. At that time a small active duodenal ulcer had been demonstrated. Gastroscopy revealed vigorous normal peristaltic waves passing over a slightly reddened and edematous antrum to a tight closure at what appeared to be a normal pylorus. The rugae on the greater curvature of the antrum were very thick. Throughout the body of the stomach there were reddening, edema and adherent secretion, with multiple petechiae and a verrucous appearance to the mucosa especially marked along the lesser curvature proximal to the angulus, where there was slight thickening. A specimen was obtained from this area for biopsy. There was no apparent rigidity or anything to suggest neoplasm. The findings were consistent with moderately severe acute superficial and chronic hypertrophic gastritis. The pathologist reported, however, that the tissue did not substantiate the diagnosis of gastritis, revealing only a normal gastric mucosa.

Comment: The pathologic report in this case was disappointing, since it did not demonstrate any gastritis in a patient with probable duodenal ulcer and roentgenographic and gastroscopic evidence of gastritis. One might argue that the radiologist and gastroscopist were both wrong in their diagnosis of gastritis, but it is more probable that the biopsy specimen was taken from a normal area in a stomach which would very likely show some degree of gastritis in other areas. In other words, the specimen was probably not a representative sample.

COMMENT

In addition to furnishing pathologic proof of the presence of gastritis, gastroscopic biopsy is certain to furnish data by means of which one may (1) study the different types of gastritis, (2) correlate the clinical, radiologic, gastroscopic and pathologic observations in gastritis [4] and (3) study the relationship of gastritis, ulcer and tumor.

Respecting superficial gastritis, in a previous report Benedict and Mallory [5] expressed the opinion that this condition as described by the gastroscopist probably corresponds to acute gastritis as described by the pathologist. By superficial gastritis the gastroscopist means reddening, edema and adherent secretion. Although 63 cases is not a large series, it is already evident that many cases of superficial gastritis are actually chronic in the opinion of the pathologist. This is in accord with Schindler's [6] use of the term "chronic superficial gastritis." In this series, up to the present time the pathologist has never reported acute gastritis alone. If acute gastritis was present, as reported, in 10 cases in this series, it has been always accompanied by chronic gastritis. Presumably, however, acute gastritis will be found unaccompanied by chronic gastritis in some cases.

Hypertrophic gastritis is reported by the gastroscopist when he sees verrucous elevations in the mucosa, sometimes with segmentation of the rugae and a dull appearance of the mucosa, with diminished highlights. Such an appearance usually corresponds to chronic gastritis as described by the pathologist. Acute changes may be superimposed. It is evident from biopsy specimens already obtained that chronic gastritis may be found pathologically when both radiologic and gastroscopic examinations fail to reveal it (see case 3). In such cases the symptoms may be explained on the basis of gastritis. It will be interesting to see whether or not most persons addicted to the consumption of alchoholic liquors actually do have some degree of gastritis as shown by gastroscopic biopsy.

Extensive intestinal metaplasia as seen histologically is one of the indications of gastric atrophy. Although there was some intestinal metaplasia in 4 cases in this series, in all of which chronic gastritis was also shown, no biopsy has been reported as demonstrating gastric atrophy. As a matter of fact, the mucosa is so thin in complete gastric atrophy that one hesitates to attempt a biopsy for fear of perforation.

Postoperative gastritis is sometimes regarded as a separate entity, but there is no gastroscopic or pathologic evidence to support this theory.

4. Benedict, E. B.: Am. J. Roentgenol. **55**:251-277, 1946.

5. Benedict, E. B., and Mallory, T. B.: Surg., Gynec. & Obst. **76**:129-135, 1943.

6. Schindler, R.: Gastroscopy, ed. 1, Chicago, University of Chicago Press, 1937.

The correlation of clinical, radiologic, gastroscopic and pathologic observations in gastritis takes careful analysis of hundreds of cases before any conclusions are justifiable. Evaluation is difficult. Gastritis may exist without symptoms, and symptoms like those of gastritis may exist without gastritis. The same is true of peptic ulcer; it may exist without symptoms, and ulcer-like symptoms may exist without ulcer. If symptoms consistent with gastritis are present and the gross gastroscopic picture is definitely that of gastritis, but the biopsy shows a normal gastric mucosa, should one rely on the gastroscopic picture or on the pathologist's report? Since positive evidence is better than negative evidence, one relies on the observation of a trained gastroscopist, discounting somewhat the pathologist's report of negative findings as being probably due to an error in sampling—meaning that the biopsy specimen may have been taken from a relatively normal area in the midst of other areas which would have revealed gastritis.

The relationship of gastritis, ulcer and cancer is not clear. Most observers agree that some gastritis accompanies every ulcer and every tumor. There is, however, a great difference of opinion as to the etiologic role of gastritis (if any) in ulcer or tumor. I have never been able to demonstrate that gastric ulcer developed in an area of gastritis. Neither has it ever been proved that carcinoma develops from gastritis, although there is some evidence that adenomatous polyps and carcinoma are more common in gastric atrophy. It is possible that frequent gastroscopic biopsy may give useful material for study regarding the precursors of ulcer and tumor.

The development and course of gastritis have never been studied. They are revealed by repeated gastroscopic biopsy. Opportunity is now presented for frequent biopsy of the stomach in the case of gastritis. Remissions and relapses can be checked by the pathologist. Although difficulties may be encountered in accurate sampling, much valuable pathologic material should be obtained.

In 6 cases in this series (10 per cent) the biopsy specimen has been considered inadequate. The decision as to adequacy is left entirely to the pathologist.

SUMMARY AND CONCLUSIONS

Routine gastroscopic biopsy has recently become practicable with the use of the flexible operating gastroscope.

Sixty-three biopsy specimens have been obtained without accident or complication.

The value of gastroscopic biopsy in the differential diagnosis of gastritis and tumor has been established.

The importance of gastroscopic biopsy in the diagnosis and study of gastritis has been pointed out.

A new field for the routine histologic and pathologic study of gastric tissues has been developed.

ABSTRACT OF DISCUSSION

Dr. H. J. Morsch, Rochester, Minn.: One deficiency of gastroscopy in the past has been the inability of the gastroscopist to remove a specimen of a suspected lesion for microscopic examination. Not infrequently the gastroscopist may obtain an excellent view of a gastric lesion but is unable to determine its exact nature. This is true especially of lesions of a diffuse nature, such as chronic gastritis, diffuse carcinomatosis and lymphomatous infiltration. It has been my experience that an error of more than 10 per cent may be anticipated in the differential diagnosis of such diffuse lesions. Very small lesions involving the gastric mucosa are likewise prone to present difficulty in differential diagnosis. In cases of these small lesions I believe it is of even greater importance than in cases of diffuse lesions to make a correct diagnosis, because if one can establish the correct diagnosis adequate medical or surgical treatment may effect a cure.

Negative biopsy observations should be accepted with reservation. In case of a suspected lesion, repeated biopsies may be required to establish the cause of the abnormality. Even then, when doubt rears its head, exploratory laparotomy may be required to settle the problem. Other procedures may be used to obtain specimens for biopsy, in addition to the operative gastroscopy. The first of these is esophagoscopy. I do not believe that esophagoscopy is used as widely as it should be in the study of gastric disease. Frequently, the gastric lesion extends into the lower end of the esophagus and a specimen for biopsy can be obtained by esophagoscopy, and microscopic examination of the specimen will establish the diagnosis. Another method is the passage of a wire spiral over a previously swallowed silk thread. Often, one is able to obtain a small specimen for biopsy in the spiral, especially in cases of diffuse lesions, which may enable one to establish a correct diagnosis. The third method, and one that I think probably will be of greater and greater importance in the study of carcinoma, is the utilization of cytology. I would like to suggest to Dr. Benedict that probably his instrument would be of even greater importance than it is now if he would develop some sort of apparatus which would enable the gastroscopist not only to obtain a specimen for biopsy but also to obtain a smear directly from the lesion for cytologic examination.

Dr. Cecil O. Patterson, Dallas, Texas: The correlation of data from adequate numbers of gastric mucosal biopsies should give much desirable new information about the stomach. Heretofore we in Dallas have studied the stomach with the flexible gastroscope when this was indicated and then introduced the esophagoscope or the longer Jackson gastroscope if a specimen was desired for biopsy. This approach has been without increase of untoward incidents and is quite satisfactory for specimens taken near the cardia. Reviewing 66 recent diagnoses of carcinoma of the stomach, I found that we obtained 11 specimens which were positive for carcinoma. The increased incidence of neoplasms near the cardia we attribute to the symptom dysphagia, because of which some of the patients sought aid. In our limited experience gastric mucosal biopsy has been helpful also when a patient has gastric distress plus anacidity and a stomach that is consistently tender to palpation at fluoroscopy. In such instances biopsies revealed extensive leukocytic and lymphocytic infiltration of an area of active acute or chronic gastritis. A 39 year old farmer complained that his stomach hurt when he walked or when he was riding his tractor, whether the stomach was filled or empty. Fluoroscopic examination revealed that the tenderness was over the antrum and the duodenal cap, and ulcer was suspected. There was no free acid on repeated histamine tests. The gastroscopic appearance was that usually described as mild chronic superficial gastritis. Several specimens of gastric mucosa were examined and further biopsies were made when symptoms recurred a year later. At both times the pathologist

reported "active acute and subacute gastritis." Another example of the value of gastric biopsy is that of a 43 year old white man, an accountant, who in 1939 was believed to have only functional gastric distress. He later had repeated massive hematemeses and an ulcer high on the lesser curvature. He was a poor subject for gastric resection, and the surgeon recommended a medical regimen. Thereupon, gastroscopic studies revealed extensive hypertrophy of the mucosa of the upper third of the stomach, very large folds being presented, with pebbling. A total of five different gastroscopic and bioptic studies were done during ten years. Although the ulcer has remained healed, biopsies have repeatedly shown extensive hypertrophy of the gastric mucosa.

I believe that gastric mucosal biopsy may prove to be justified in patients having less specific gastric distress, as, for example, in those with disorders of nutrition or of metabolism or those with endocrine disorders, as well as in those with inflammatory conditions of the stomach, or with the changes of age or changes of the emotions or in the study of the postoperative stomach, or of the genesis of neoplasia within the gastric mucosa. No doubt every one here can recall conditions in which biopsy might have been of value. Gastric mucosal biopsy provides probably the most satisfactory way in which the microscopic gastric pathologic changes may be studied from the time of the earliest symptoms to the later course of a gastric disease in the living patient.

DR. LOUIS E. MARSHALL, Manhasset, N. Y.: As a practicing pathologist I think this would be a fitting opportunity, in a joint meeting, to suggest that the percentage of specimens of the stomach taken for biopsy in cases of cancer might be increased.

Dr. Moersch has suggested the development of a gadget which would increase the percentage of positive results of biopsies, and I should like to mention an additional method by which one may increase this percentage. Dr. Sidney A. Gladstone, professor of pathology at the New York Polyclinic Medical School and Hospital, has done a great deal in this respect by introducing a sponge biopsy into the diagnosis of cancer. This consists in the use of a piece of gelfoam® which may be rubbed over the surface of any lesion that may be exfoliating cancer cells. He presented this method for the first time in 1948, and subsequently at the convention of the American Society of Clinical Pathologists. He has a scientific exhibit at this convention, also. I believe that if a piece of this gelfoam® could be applied to the end of the operating gastroscope, there might be a greater possibility of increasing the percentage of positive diagnoses in those cases in which it is difficult to take numerous specimens for biopsies. Dr. Gladstone recommends that the sponge be rubbed over any surface that arouses suspicion and that this sponge specimen can then be sent through the regular procedure, that is, formaldehyde fixation and the making of paraffin sections. Both sides of the sponge may be exposed to the ulcerative lesion, and if the pathologist does not get a positive section on one surface, he turns it over and cuts sections from the other surface. Of course, the pathologist would still like to have a tissue specimen on which to base his diagnosis, but I suggest the sponge procedure as one whereby the percentage of gastric biopsies that fail to yield evidence of cancer may be decreased.

DR. EDWARD B. BENEDICT, Boston: I agree with everything Drs. Moersch, Patterson and Marshall have said. I went to see Dr. Gladstone yesterday at his exhibit, and took him around to see the operating gastroscope, with the idea that we might be able to get some kind of sponge biopsy by passing a sponge through the operating channel. That is something for the future.

DISTRIBUTION OF LIPASE IN PRENEOPLASTIC AND NEOPLASTIC STATES INDUCED IN THE RAT LIVER BY PARADIMETHYLAMINOAZOBENZENE

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THAT THERE exists in tissues a class of enzymes capable of hydrolyzing esters of fatty acids has long been known.[1] Wells,[2] in a review of the literature, stated that enzymes splitting ethyl butyrate and other lower, short chain, fatty acid esters had been demonstrated in most tissues examined. Technical difficulties, however, i. e., the insolubility of the esters of the higher, naturally occurring fatty acids, have prohibited precise investigation of the enzyme splitting neutral fats.

While the role of the lipase hydrolyzing the natural esters of the higher fatty acids remains obscure, some evidence has been accumulated about the ability of normal and pathologic tissues to cleave butyrates. Winternitz and Meloy,[3] on the basis of chemical (titrimetric) determinations, showed that an increase of butyrase activity occurred in various tissues as cells matured beyond the embryonic state; lower than normal enzyme levels were observed in cirrhosis of the liver.

A cytochemical method recently proposed by Gomori [4] for the determination of lipase activity utilizes as substrate for the enzyme one of several higher fatty acid esters of mannitan or of sorbitan.[5] These materials are water soluble, and are apparently innocuous when given orally or parenterally to experimental animals.[6] Employing these

From the Laboratories of the Rockefeller Institute for Medical Research.

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* Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

1. Hanriot, M.: Compt. rend. Soc. de biol. **49**:377, 1897.

2. Wells, H. G.: Chemical Pathology, ed. 4, Philadelphia, W. B. Saunders Company, 1920.

3. Winternitz, M. C., and Meloy, C. R.: J. M. Research **22**:107, 1910.

4. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **58**:362, 1945.

5. These compounds are manufactured by the Atlas Powder Company, Wilmington, Del., and are marketed with the names "product G-9096 CJ" or "tweens 20,40,60 or 80."

6. Mark, D. D.: Unpublished observations.

esters in a neutral buffered medium, Gomori detected and localized histologically the sites of lipase activity in tissue sections. Archibald[7] has outlined a method for the quantitative determination of lipase activity in which "tween 20," an aqueous solution of sorbitan monolaurate (polyoxyalkalene derivative), is used as substrate.

In the present report the distribution of lipase is described as observed in precancerous and cancerous lesions of the liver induced in white rats with paradimethylaminoazobenzene ("butter yellow"). This substance produces an orderly sequence of pathologic changes in the liver, beginning with fatty degeneration; later the proliferation of bile ducts, liver cells and supporting fibrous tissue, which terminates in the appearance of tumors, provides an opportunity to study lipase in the cytoplasm of the tumor cells and their antecedents.

MATERIAL AND METHODS

The characteristic hepatic changes recorded are essentially those described by Opie,[8] and they were elicited by paradimethylaminoazobenzene in white rats given a diet of brown rice and carrots (Kinosita[9]). The paradimethylaminoazobenzene was added to the diet in the proportion of 0.06 per cent, 20 cc. of a 3 per cent solution of the dye in olive oil being added to 1,000 Gm. of the diet. Animals on this regimen and control animals were killed at frequent intervals within a period between one-half month and six months after the beginning of the experiments. Liver blocks were fixed in 4 per cent solution of formaldehyde.

The histologic localization of lipase outlined by Gomori[10] was adapted to the study of paraffin sections after the tissue had been fixed in acetone. Several observers, however, have found that the processes of fixation and embedding of the tissue may often destroy the enzyme. In this study, more consistent results and sections of better quality were obtained with frozen sections of the formaldehyde-fixed tissue blocks. In addition, the relation of lipase and fat in the liver could be delineated, whenever necessary, by staining with sudan IV and hematoxylin after the lipase reaction had been completed.

Frozen sections of liver were incubated at 37 C. for three hours in a substrate mixture of the following composition: 5 cc. of stock solution 1 (5 per cent "tween 60" or "product G-9096 CJ")[11] and 95 cc of stock solution 2 (50 cc. of 10 per cent calcium chloride, 50 cc. of sodium barbital buffer at p_H 7.4, 150 cc. of glycerin and distilled water to make 1,000 cc.). The sections were then rinsed in water, placed in 2 per cent lead nitrate for fifteen minutes, rinsed again and transferred to dilute ammonium sulfide for two minutes. By these manipulations, the calcium in the soap precipitated at the presumptive site of lipase activity was supplanted by the lead, which was then demonstrated as the insoluble brown sulfide. Some brown precipitate, indicating the site of the enzyme, was present in all the sections

7. Archibald, R. M.: J. Biol. Chem. **165**:443, 1946.

8. Opie, E. L.: J. Exper. Med. **80**:231, 1944.

9. Kinosita, R.: Tr. Jap. Path. Soc. **27**:665. 1937.

10. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **58**:362, 1945.

11. "Tween 60" and "product G-9096 CJ" are stearic esters of sorbitan and mannitan, respectively.

examined. The precipitate appeared as large brown granules in the cytoplasm of the liver cells. Control sections incubated in the substrate mixture devoid of "tween" product, and otherwise similarly treated, showed no brown precipitate. Reactions to the test were elicited in sections from tissue blocks preserved in formaldehyde solution as long as three months. It is noteworthy that pigment granules which give the prussian blue reaction for ferric iron form a black precipitate of iron sulfide.

NORMAL LIVER

In the normal liver of the rat the distribution of the sites of lipase activity, denoted by a brown precipitate, is definite and constant. In the cells in the area around the terminal ducts, that is, in the portal spaces, the precipitate is in moderate quantity, but the cells in the region of the central veins are densely brown, indicating a larger share in the lipase activity of the lobule (fig. 1 *A*).

Following the administration of paradimethylaminoazobenzene, the earliest demonstrable change has been the conspicuous absence of brown precipitate in the cytoplasm of the cells in the region of the portal spaces, although the intensity of the reaction in the areas around the hepatic (central) veins remains essentially unchanged (fig. 1 *B*). The sites of early edema of the vascular septums that define portal units (Mall [12]) are clearly delineated by the lipase reaction. Cells at the periphery of the unit react strongly, but those more centrally placed show a gradually diminishing reaction as the portal space is approached (fig. 1 *C*).

CIRRHOSIS

When the livers of the animals that have received paradimethylaminoazobenzene are examined within the first few months of its administration, fatty degeneration may be evident in the parenchymatous cells surrounding the hepatic (central) veins; in some instances these cells may have disintegrated and disappeared. With progression of these lesions, changes are found in the portal areas, and ultimately they terminate in characteristic nodular cirrhosis. The hepatic lobule is then greatly distorted by bands of disintegrating cells, accumulations of mononuclear phagocytes and proliferations of fibroblasts, which may unite periportal tissue with that about hepatic veins. These changes often surround islands of parenchymatous hyperplasia.

In the cytoplasm of cells about central veins, where fat is first seen to accumulate, the lipase reaction is absent. As the process of fibrosis advances, no lipase activity is demonstrable in the fibrous tissue. Nevertheless, peripherally in the islands of cells that have survived the destruction of liver tissue the lipase reaction is particularly intense, while in the centers of these nodules it is found in gradually diminishing

12. Mall, F. P.: Am. J. Anat. 5:227, 1906.

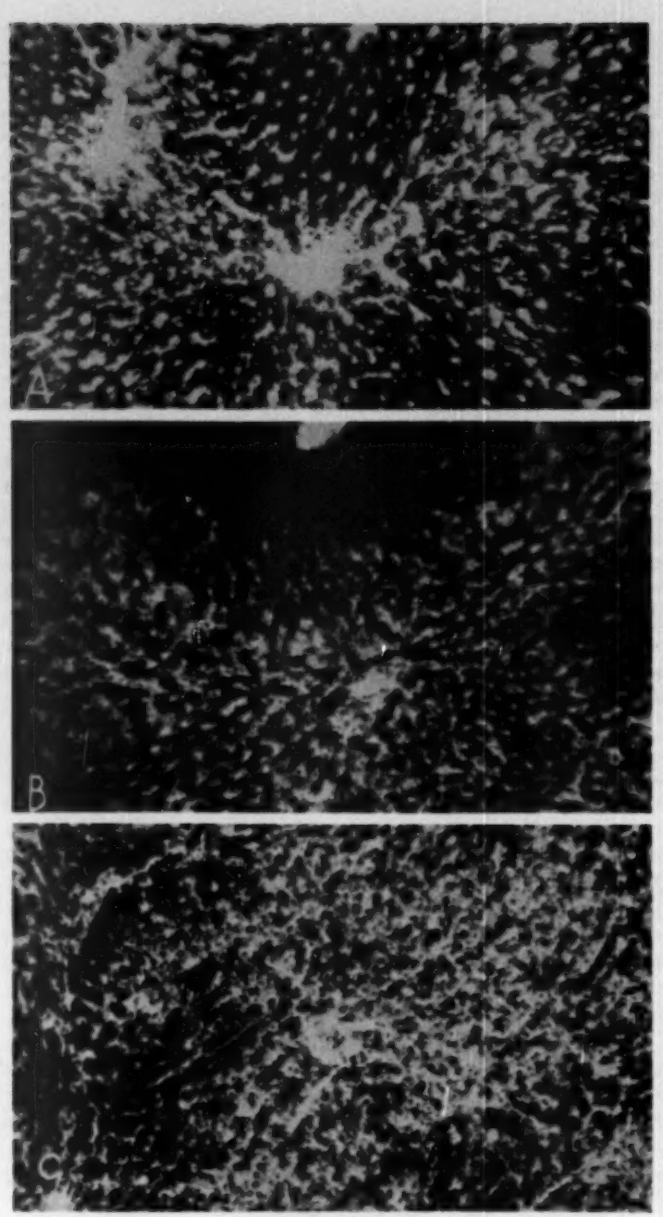

Figure 1

(See legend on opposite page)

intensity (fig. 2 *A*). Fat, stained by sudan IV, may sometimes be seen lying just within the zone of the most intense lipase reaction.

HYPERPLASIA OF LIVER CELLS

Hyperplasia of liver cells induced by paradimethylaminoazobenzene usually appears during the third to fifth month of feeding. Hyperplasia may be diffuse without sharp demarcation, or in foci, so that rounded, circumscribed nodules of liver cells are formed. Neither type bears any constant relation to the lobular structure of the liver. The enlarged hyperplastic cells have large nuclei and are sometimes arranged to form tubular structures.

The liver parenchyma bordering an area of diffuse hyperplasia may be sharply demarcated by the lipase reaction, in contrast to the hyperplastic cells which are almost devoid of evidence of lipase activity (fig. 2 *B*). In foci of circumscribed hyperplasia the cells are equally lacking in enzyme activity, particularly where the cells are enlarged and their nuclei hyperchromatic. Often this is in contrast with the nodular islands of parenchyma seen with cirrhosis, for in these the cells sometimes show a normal or even heightened lipase reaction.

FORMATION OF NEW BILE DUCTS AND CYSTIC DUCTS

In the earlier stages of cirrhosis, newly formed bile ducts may be found in the new connective tissue. These ducts are tubular structures with cubical epithelium resembling terminal bile ducts, and are seen about portal spaces, near central veins or in the intermediate connective tissue. At a later stage, cystic ducts, which appear as tortuous, dilated channels with flat epithelium, are apparently derived from the new bile ducts.

In all these areas there is no reaction for lipase in the duct epithelium and fat is not seen (fig. 2 *C*).

EXPLANATION OF FIGURE 1

Fig. 1.—All the photographs show frozen sections of formaldehyde-fixed liver. Magnification, × 270.

A, normal rat liver. The precipitate of the lipase reaction is found in the cytoplasm of the hepatic cells. It is in moderate quantity in cells around the portal spaces (center and top left), but in cells around central veins (top center) the precipitation is most intense. The nuclei are unstained.

B, rat liver after two weeks' administration of paradimethylaminoazobenzene. The precipitate of the lipase reaction is greatly diminished in the cells in the region of the portal space (center), while it remains unchanged in cells near the central vein (top center). The nuclei are stained with hematoxylin.

C, rat liver after two months' administration of paradimethylaminoazobenzene. Note the delineation of a portal unit (Mall) by the lipase reaction. A zone of heavy precipitation borders the vascular septums at the periphery of the unit. Cells near the centrally placed portal space show less precipitate. The nuclei are stained with hematoxylin.

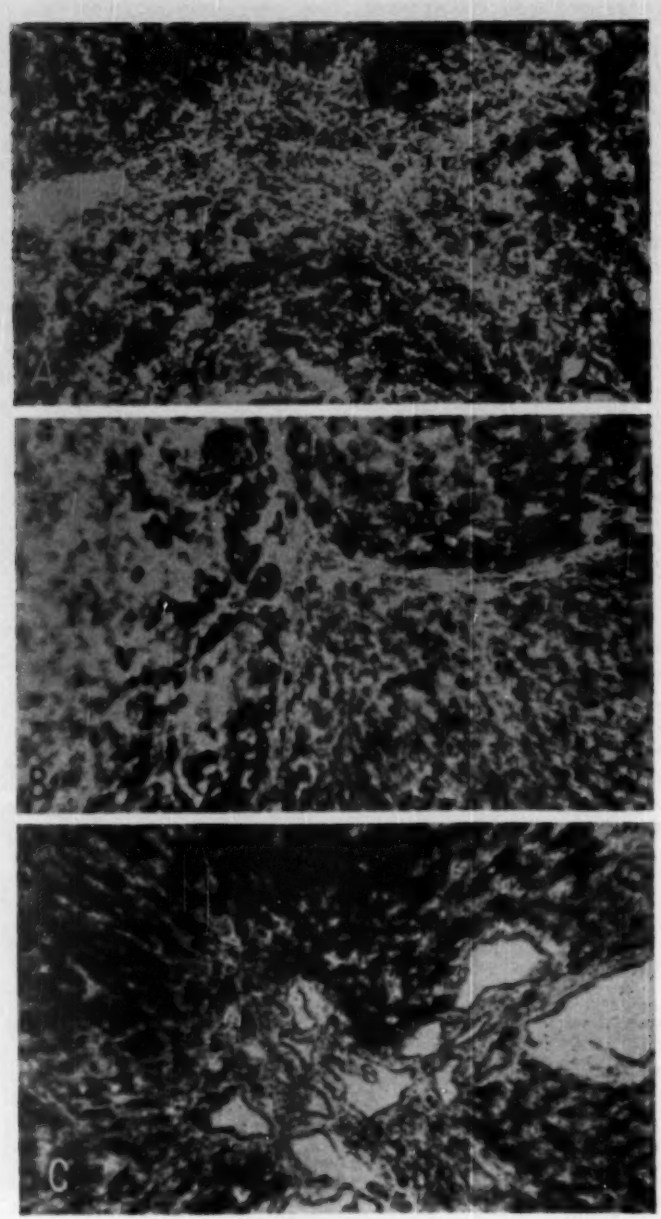

Figure 2

(See legend on opposite page)

CHOLANGIOFIBROSIS

In the livers of the rats which have ingested paradimethylaminoazobenzene for three to five months, isolated foci of bile duct proliferation develop which are associated with the formation of dense fibrous tissue. This change often precedes the development of tumors. The epithelium of the newly formed tubules is cubical, and the widely dilated lumens may contain a mucinous material. These foci of cholangiofibrosis are often in immediate contact with portal spaces.

The fibrous tissue, the cubical epithelium and the contents of the tubules give no reaction for lipase, although it is conspicuous in the adjacent parenchyma.

HEPATOMA AND CHOLANGIOMA

Focal hyperplasia, cystic ducts and cholangiofibrosis, the antecedents of the hepatic tumors produced by paradimethylaminoazobenzene, may exhibit some of the gross and microscopic aspects of cancers. The tumors arising from them have evident capacity for autonomous propagation. Hepatoma arises from foci of hyperplastic liver cells, cystadenoma from cystic ducts and cholangioma from foci of cholangiofibrosis. Metastases with the morphologic characters of the primary growth are found with each type. Hepatoma, composed of anastomosing cells still recognizable as liver cells, or of tubules or glandlike structures formed by similar cells, occurs as more or less sharply defined, soft, yellow nodules, often attaining a diameter of 2 cm. or more. The cytoplasm of the cells in these nodules shows no evidence of lipase activity. Occasional islands of cells that give the lipase reaction may sometimes be seen around the fringes of the nodules, but it is doubtful if these cells belong to the neoplasm (fig. 3).

Highly proliferative cystic structures forming papillary projections (cystadenoma), as well as tumors composed of irregular glandular

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EXPLANATION OF FIGURE 2

Fig. 2.—All the photographs show frozen sections of formaldehyde-fixed liver. Magnification, × 270.

A, cirrhosis of the liver of a rat after three months' administration of paradimethylaminoazobenzene. No lipase reaction is demonstrated in the fibrous tissue (center); however, the precipitation is intense at the periphery of an island of hepatic cells (lower center). The nuclei are stained with hematoxylin.

B, hyperplasia of hepatic cells in rat liver. There is no precipitate indicating lipase activity in the area of hyperplasia (lower right). In the cells of the cirrhotic nodules which appear above this point, the lipase activity is found in the peripheral cells (as in *A*). The nuclei are stained with hematoxylin.

C, newly formed bile ducts in the liver of a rat after three months' administration of paradimethylaminoazobenzene. There is no precipitate indicating lipase activity in the cells of the newly formed bile ducts (center). The nuclei are stained with hematoxylin.

alveoli bearing hyperchromatic nuclei (cholangioma), may also be distinguished. The actively growing low cubical cells of the former and the polygonal cells of the latter, bearing a clear resemblance to cystic ducts and to the ducts of cholangiofibrosis, respectively, show no brown cytoplasmic precipitate indicative of lipase activity.

COMMENT

Paradimethylaminoazobenzene continuously administered in a rice-carrot diet induces in the liver cells of rats a series of changes which progress in an orderly manner to tumor formation. The tumor antecedents, namely, focal hyperplasia of liver cells, cystic ducts and cholangiofibrosis, and the tumors derived from them, are accompanied

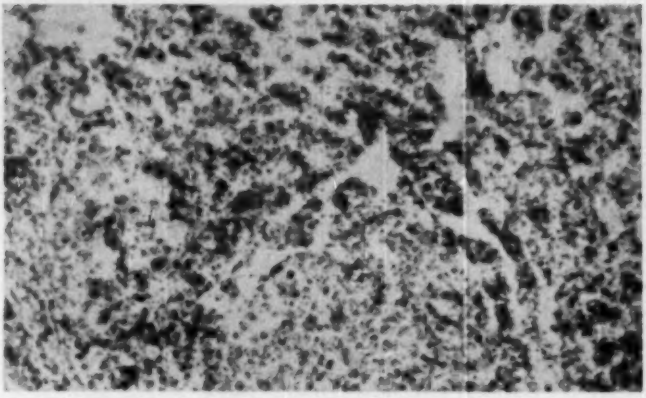

Fig. 3.—Hepatoma of the liver of a rat (frozen section of formaldehyde-fixed tissue; × 270). The body of the tumor shows no lipase reaction. There are occasional islands of persisting liver cells showing the precipitate of the lipase reaction on the left fringe of the tumor. The nuclei are stained with hematoxylin.

by chemical changes reflected in a decline or loss of the lipolytic activity of the cytoplasm. Occasionally, islands of liver cells associated with cirrhosis of the liver may be distinguished by normal or heightened lipase activity.

The observations of the weak capacity for hydrolyzing higher carboxylic esters, possessed by the cells of the precancerous and cancerous lesions that have been described, are in accord with the findings of the low capacity for hydrolyzing butyrates demonstrated in vitro for various mouse tumors by Greenstein.[13] No information exists concerning the total lipase activity of the rat liver after the administration

13. Greenstein, J. P.: J. Nat. Cancer Inst. 5:31. 1944

of the toxic agent paradimethylaminoazobenzene, although after the administration of the other hepatotoxic agents, such as chloroform (Quinan [14]), the total liver lipolytic (butyrase) activity was found to be depressed, while after phosphorus poisoning (Loewenhardt [15]) there was no perceptible change.

The lipolytic enzyme demonstrated in the liver by these investigations is presumably capable of hydrolyzing not only the carboxylic esters studied but the naturally occurring triglycerides as well. The same enzyme may also catalyze the reverse reaction, namely, the synthesis of fats from fatty acid and glycerin.[15] In the sections studied, however, no lipase activity is demonstrated in regions of the liver where fat accumulates, and the distribution of the fat bears no evident relation to the lesions observed.

In the detection and localization of the lipase it is apparent that formaldehyde fixation of the liver does not seriously impair its capacity for splitting fatty acid esters. Koch [16] found that formaldehyde did not destroy lipase. On the contrary, Nachlas and Seligman [17] reported a loss of as much as 60 per cent of the original lipolytic power of a comparable enzyme (esterase) following acetone fixation and embedding procedures.

The specificity of the Gomori reaction [18] as a method of demonstrating purely lipase activity has been questioned. Although the enzyme demonstrated by the reaction acts on fatty acid esters, it has limited specificity, hydrolyzing substrates with varying chemical configurations. Furthermore, differences of various activators and inhibitors of the enzyme have been found in different tissues.[18] It is probable that a group of related enzymes, not as yet defined, may account for some of these observations. Many observers, including Baldwin,[19] have designated the enzymes hydrolyzing carboxylic esters as esterases.

SUMMARY

A method designed for the detection of lipase in tissue sections and using a stearic ester of sorbitan or of mannitan as substrate has been applied to the study of the distribution of lipase activity in the liver of (1) rats fed a stock diet or a rice and carrot diet (controls) and (2) rats fed a rice and carrot diet containing paradimethylaminoazobenzene ("butter yellow").

14. Quinan, C.: J. M. Research **32**:73, 1915.
15. Loewenhardt, A. S.: Am. J. Physiol. **6**:331, 1902.
16. Koch, W.: Am. J. Physiol. **6**:325, 1902.
17. Nachlas, M. N., and Seligman, A. M.: J. Nat. Cancer Inst. **9**:415, 1949.
18. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **77**:4, 1948.
19. Baldwin, E.: Dynamic Aspects of Biochemistry, London, Cambridge University Press, 1947.

In the livers of the rats fed the control diet, lipase activity was found in the cytoplasm of the hepatic cells, and appeared more concentrated in cells about the central veins than in those about the portal spaces. The bile duct cells were devoid of any lipase activity.

In the livers of the rats fed the diet containing paradimethylaminoazobenzene the observations were as follows: 1. No reaction indicating lipase activity was detected in localized areas of advanced fatty degeneration, in the cells of proliferating bile ducts or in the cells of cancerous hepatoma. 2. In areas of hyperplasia of hepatic cells the lipase activity was weak or absent.

The results of the experiments suggest that the changes produced in the rat liver by paradimethylaminoazobenzene coincide with a weakening or a complete disappearance of lipase activity in lesions, precancerous and cancerous, which appear to be derived from hepatic cells.

INCIPIENT SQUAMOUS CELL CARCINOMA OF CERVIX UTERI

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AND
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DETECTION of carcinoma of the cervix uteri has gained momentum through the popularization of Papanicolaou's method [1]; yet in the reported series of cases those of carcinoma in the incipient stage have not exceeded 2 per cent of all cases of squamous cell carcinoma of the cervix uteri.[2] A survey of recent records at the University of Oklahoma Hospitals disclosed that in 10 per cent of the cases of carcinoma of the cervix uteri the carcinoma was in the incipient stage. This increase in incidence of incipient carcinoma can be attributed to systematic studies of spreads of cells from the line of junction between the squamous and the columnar epithelium of the cervix uteri.[3] These were made for patients in the admitting clinic who had no gynecologic symptoms, for patients in whom a cervical biopsy was indicated, and for patients in whom there were indications for hysterectomy. The cases of incipient growth were analyzed as to ages of the patients, relative frequency in different decades of life, and clinical and microscopic features.

MATERIAL AND METHOD

Between Jan. 1, 1946 and Aug. 31, 1949, at the University of Oklahoma Hospitals, a positive diagnosis of carcinoma of the cervix uteri was made by histologic examination in 275 cases. In 28 of these the squamous cell carcinoma of the cervix uteri was in the incipient stage. In 12 of these total hysterectomy, and in 1 excision of the cervical stump, made the entire cervix uteri available for microscopic study.

From the Departments of Pathology and Gynecology, University of Oklahoma School of Medicine, and the University of Oklahoma Hospitals.

1. Papanicolaou, G. N., and Traut, H. F.: Diagnosis of Uterine Cancer by the Vaginal Smear, New York, The Commonwealth Fund, 1943.

2. Cox, K. E.; Buhler, V. B., and Mixson, W. C.: Am. J. Obst. & Gynec. **56**:112, 1949. Galvin, G. A., and TeLinde, R. W.: Am. J. Obst. & Gynec. **57**:15, 1949.

3. Ayre, J. E.: Am. J. Obst. & Gynec. **53**:609, 1947.

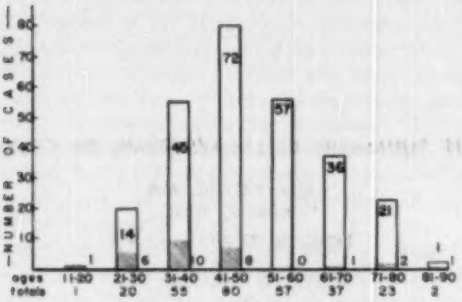

Fig. 1.—Age incidence of carcinoma of the cervix uteri (incipient carcinoma shaded).

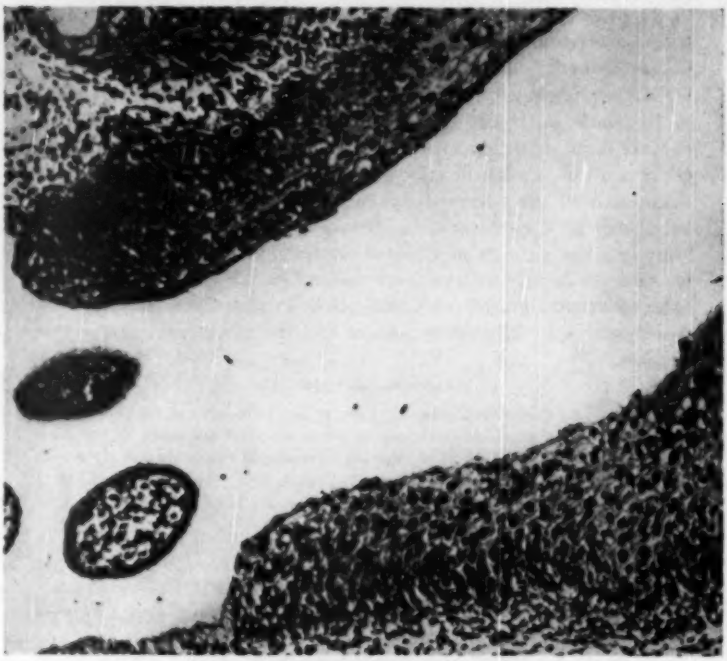

Fig. 2.—Incipient squamous cell carcinoma of the cervix uteri; × 200. The patient, a 33 year old Negro woman, had been pregnant twice and had given birth to 2 children. She complained of postcoital bleeding and foul discharge for one month. The cervix showed a slight erosion in the posterior lip. Biopsy revealed early squamous cell carcinoma of the cervix uteri.

INCIDENCE

Of the 275 patients (210 white and 65 Negro) with carcinoma of the cervix uteri, the youngest was 19 and the oldest 81 years old. The peak of incidence was in the fifth decade. The incidence was almost alike in the preceding (fourth) and in the subsequent (sixth) decade (fig. 1). Of the 28 patients (24 white and 4 Negro) with early carcinoma, the youngest was 22 and the oldest 81 years old. Among these, 6 were in the third, 10 in the fourth, 8 in the fifth, 1 in the seventh, 2 in the eighth and 1 in the ninth decade of life. Thus the peak of incidence of early carcinoma of the cervix uteri was in the fourth decade.

Fig. 3.—Incipient squamous cell carcinoma of the cervix uteri; × 200. The patient, a 29 year old white woman, had been pregnant eight times and had given birth to 7 children. Her complaint could be referred to a cystocele and a rectocele. Spreads from the squamous-columnar cell junction of the cervix uteri disclosed no neoplastic cells. Biopsy of an eroded area revealed early carcinoma. Two months later another biopsy disclosed that the carcinoma was invading the deeper layers.

CLINICAL FEATURES

Among the 28 patients with early carcinoma of the cervix uteri, 14 had no signs or symptoms that could be referred to the cervix uteri. The remaining 14 com-

plained of abnormal bleeding. Direct inspection of the cervix revealed the normal appearance somewhat altered in all but 2 patients. The slight changes ranged from minute focal red discoloration to erosion of part or most of the circumference of the external os. In each patient the lesion had the appearance of a chronic inflammatory process rather than that of a cancerous growth. A cytologic examination of spreads from the squamous-columnar cell junction was made in 19 cases, in 15 of which abnormal cells were recognized.

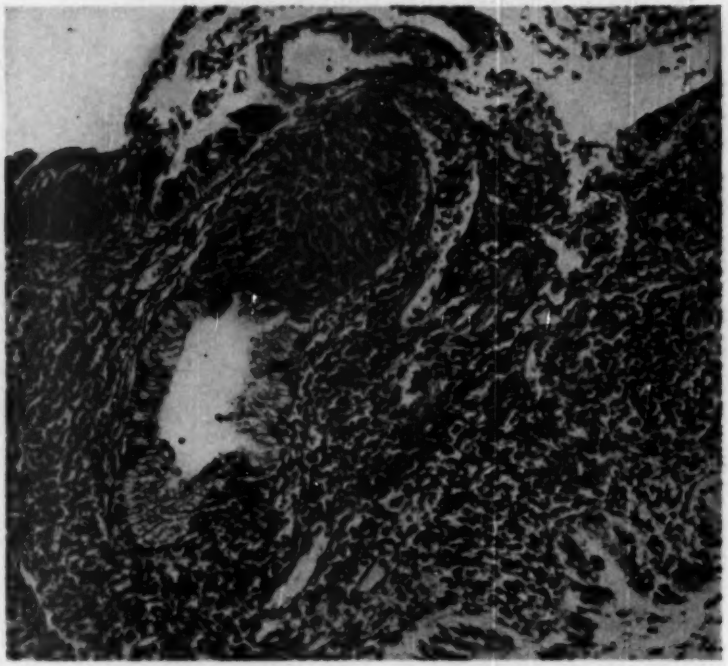

Fig. 4.—Early squamous cell carcinoma of the cervix uteri; × 200. The patient, a 35 year old white woman, had been pregnant eight times and had given birth to 7 children. Vaginal hysterectomy was performed because of cystocele, rectocele, chronic cervicitis and menometrorrhagia. Spreads from the squamous-columnar cell junction of the cervix uteri showed cancer cells. Microscopic examination of the cervix uteri revealed early squamous cell carcinoma.

MICROSCOPIC FEATURES

An incipient or early carcinoma of the cervix uteri is not discernible grossly. It may be defined as one in which the neoplastic squamous epithelial cells are on the surface (figs. 2 and 3) or immediately beneath the surface (figs. 4, 5 and 6).

The growth is still in situ; that is, its extension is on the surface and is limited to the tissues immediately subjacent to the covering epithelium.[4] The criteria for designating the cells as neoplastic pertain to the individual and group characteristics of the cells, to the relation of the neoplastic cells and the stroma and to the site of their occurrence.

Useful individual characteristics are: the deeper staining of the neoplastic cells, which are thus in contrast to nearby non-neoplastic cells; the increased size of the cells and of their nuclei (fig. 6); the variation in size of cells and nuclei; the

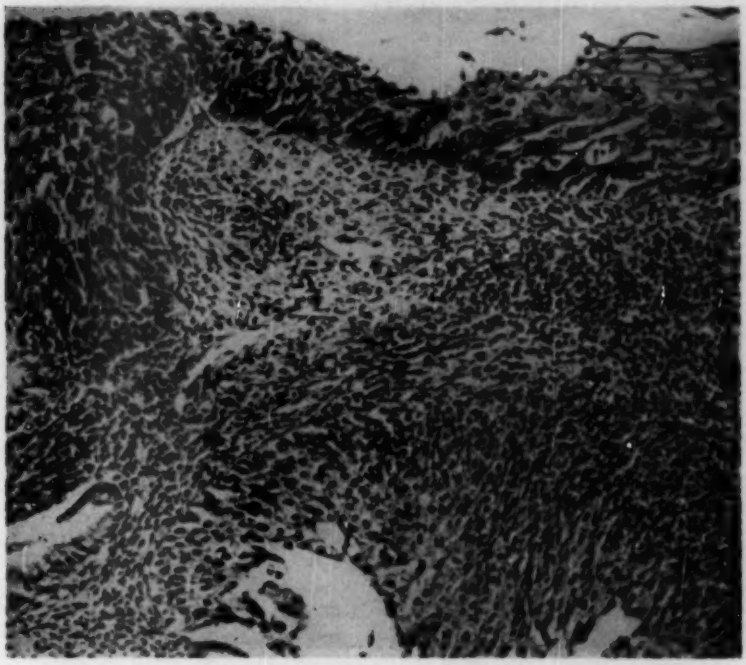

Fig. 5.—Early squamous cell carcinoma of the cervix uteri; × 200. The patient, a 38 year old Negro woman, had been pregnant five times and had given girth to 3 children. She had no gynecologic signs or symptoms. Spreads from the squamous-columnar cell junction of the cervix uteri revealed cancer cells. Biopsy of a slightly reddened area of the cervix disclosed early squamous cell carcinoma. Radical hysterectomy with bilateral salpingo-oophorectomy was performed. Microscopic examination of the cervix uteri revealed early carcinoma.

increased density of the nuclei (figs. 4 and 5); the clearly discernible nucleoli (fig. 6), and the presence of an increased number of cells in typical and atypical

4. Schiller, W., in Anderson, W. A. D.: Pathology, St. Louis, C. V. Mosby Company, 1948, p. 1135. Foote, F. W., Jr., and Stewart, F. W.: Cancer **1**:431, 1948. Lock, F. R., and Caldwell, J. B.: Am. J. Obst. & Gynec. **57**:1133, 1949.

cell division. Among the group characteristics the lack of palisading of the basal layer, the widening of the intermediate layer, the irregularity of the keratinized layer and the change in pattern of the layering of the cells are useful (figs. 3 and 4). In most instances in the present series the layer of neoplastic cells was broad, two to five times the usual width of the squamous epithelium (figs. 2, 3 and 6). The line of transition between the neoplastic and non-neoplastic cells was frequently almost straight and was oblique or perpendicular to the surface.[5] An inconspicuous, newly formed stroma supported the neoplastic cells. Beneath the neoplastic cells

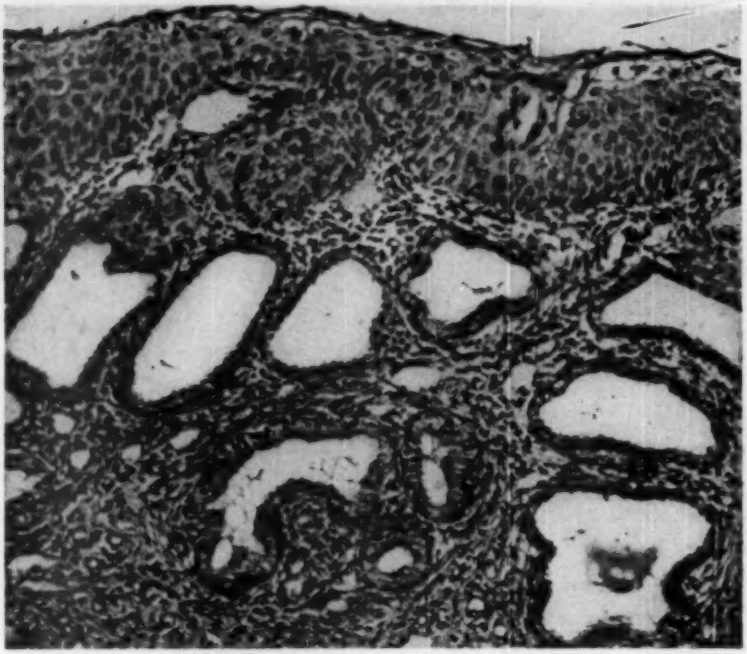

Fig. 6.—Incipient squamous cell carcinoma of the cervix uteri; × 200. The patient, a 22 year old white woman, had had no pregnancies or miscarriages. A biopsy specimen taken from a small reddened area of the cervix uteri in the course of treatment for Trichomonas vaginalis infection disclosed early squamous cell carcinoma of the cervix uteri.

or surrounding them the connective tissue stroma was often infiltrated with lymphocytes, plasma cells, large mononuclear cells and some granulocytes (figs. 4 and 5).

The usual site of the neoplastic involvement of the cervix uteri appeared to be along the junction of the squamous and the columnar epithelium. Usually the

5. Gurskis, E. E.; Beaver, D. C., and Nelson, H. M.: Surg., Gynec. & Obst. **85**:727, 1947.

neoplastic squamous epithelial cells extended beyond the terrain covered by squamous epithelium into areas normally covered by columnar epithelium (figs. 3 and 6). Cervical glands frequently opened on the surface covered by neoplastic squamous epithelium (fig. 2). The neoplastic cells often extended along the openings of cervical glands, replacing the usual columnar cells (figs. 2 and 4), or formed deep epithelial ridges, or were seen as solid cell nests with individual cell keratinization in the center (fig. 5). Dipping of the neoplastic epithelial cell nests beneath the surface occurred frequently (figs. 4 and 5).

One often observes squamous epithelial cells extending over areas usually covered by columnar epithelium and along the openings of cervical glands in chronic cervicitis. This extension and overgrowth of the squamous epithelium is a part of the chronic inflammatory reaction and is to be distinguished from similar behavior of cancer cells.

COMMENT

The characteristic microscopic features just enumerated establish the existence of a distinct incipient stage of carcinoma of the cervix uteri. At this stage the carcinoma frequently gives rise to no clinical symptoms and presents no characteristic gross appearances. It may be suspected by the presumptive evidence supplied by examination of spreads of cells from the vaginal vault or from the squamous-columnar cell junction of the cervix uteri, and can be diagnosed by microscopic examination of a biopsy specimen.

In the cervix uteri, when neoplastic epithelial cells first appear in continuity with the intact squamous epithelium, they displace, replace or grow away from the non-neoplastic epithelium. Since these neoplastic cells are not contained within the preexisting covering epithelium, they are not intraepithelial. Since the growth usually spreads over a new terrain normally covered by columnar epithelium, the growth is invasive and is not in situ. Thus, in our opinion an incipient carcinoma of the cervix uteri is not intraepithelial, is not noninvasive and is not in situ. The term "in situ" when used correctly indicates that the growth, as far as can be ascertained, is still on the surface and has not extended beyond the tissues immediately subjacent to the covering epithelium of the cervix uteri.

Conclusive evidence as to how long incipient carcinomas may remain clinically latent is not as yet available. The impression of some investigators that it occurs in the younger age group [6] and that it may remain latent for half a decade or longer remains a matter of opinion.[6] The age incidence of early carcinoma of the cervix uteri perhaps is not unlike that of carcinoma in the further advanced stages.[7] In 24 of the

6. Diddle, A. W.; Ashworth, C. T.; Brown, W. W., Jr., and Bronstad, M. T., Jr.: Am. J. Obst. & Gynec. **57**:376, 1949. Procails, A. B., and Campbell, D. A.: J. Michigan M. Soc. **48**:309, 1949. Cox and others.[2]

7. Sadugor, M., and Palmer, J. P.: Am. J. Obst. & Gynec. **56**:680, 1948.

28 cases in our series it occurred in the third, fourth and fifth decades; in the remaining 4, however, it occurred in the seventh, eighth and ninth decades.

SUMMARY AND CONCLUSION

An incipient squamous cell carcinoma of the cervix uteri is defined as one in which the neoplastic squamous epithelial cells are on, or immediately beneath, the surface, their extension being limited to the tissues immediately subjacent to the surface. Studies are presented of 28 cases of incipient carcinoma of the cervix uteri observed among 275 cases in which a positive diagnosis of carcinoma of the cervix uteri was made by histologic examination. The data obtained reemphasize the fact that carcinoma of the cervix uteri is detectable in its incipient stage when clinical signs and symptoms are wanting and while the lesion is still local. Detection of such lesions is important, because it is likely they can be cured by methods of treatment now available.

ONKOCYTES AND THE SO-CALLED HÜRTHLE CELL TUMOR

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A PECULIAR type of cell occurring in the salivary gland has been called an onkocyte.[1] The cell has been observed in certain tumors of this gland,[2] sometimes forming the epithelial part of adenolymphoma and at other times pure adenoma. These observations have been generally confirmed, and Jaffé recommended the name "onkocytoma" for the tumors of the salivary gland composed entirely of these cells.[3] Nevertheless, the recognition of onkocytes is still difficult because so far no specific stain has been available.

Feyrter[4] developed a specific staining technic very suitable for a simple and clear demonstration of onkocytes. The so-called inclusion staining is performed as follows:

A frozen section of material fixed in formaldehyde solution (formalin®) is mounted from distilled water onto a slide. The water is removed, and the slide is covered with the staining solution. In contrast to other technics, the staining solution is not removed, but the slide is immediately covered with a cover glass which is surrounded with a sealing medium. Since the slide remains included in the staining solution, the term "inclusion stain" has been introduced. The sealing prevents the staining solution from drying out, so that properly prepared slides are stable for years. The staining effect does not take place immediately but only after several hours. A 1 per cent thionine or cresyl violet solution[5] in 0.5 per cent aqueous tartaric acid is used. With the thionine inclusions stain, mucus, certain lipids which are otherwise not demonstrable, myelinated and nonmyelinated nerves and other tissues reveal red to pale pink metachromasia. Certain lipids, e. g. cardiolipid, show blue metachromasia with cresyl violet.

The onkocytes are clearly demonstrated by staining with either dye, because of their metachromasia. This property is apparently due to certain lipids which they contain and which give only a pale noncharac-

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From the Pathological Institute of the University of Marburg/Lahn.

1. Hamperl, H.: Ztschr. f. mikr.-anat. Forsch. **27**:1, 1931.

2. Hamperl, H.: Virchows Arch. f. path. Anat. **282**:724, 1931.

3. Jaffé, R.: Am. J. Cancer **16**:1415, 1932. Ackermann, L. V.: Arch. Path. **36**:508, 1943.

4. Feyrter, F.: Virchows Arch. f. path. Anat. **296**:645, 1936; Ztschr. f. mikr.-anat. Forsch. **51**:610, 1942; Wien. Ztschr. f. inn. Med. **28**:5, 1947.

5. Feyrter, F.: Mikroskopie (Wien) **1**:49, 1946.

teristic color with the sudan stain. Inclusion staining permits simple demonstration of the onkocytes, and in questionable instances the decision as to whether a cell is an onkocyte or not.

Prior to application of the inclusion stain it had been already claimed [6] that onkocytes occur not only in salivary glands but also in other epithelial organs. This claim was based on the appearance of the cells revealed with the usual stains. Use of the inclusion stain confirms this opinion, as the onkocytes of other glands show the same metachromasia as those of the salivary glands. Onkocytes occur not only in the salivary glands but also in the thyroid, parathyroid, pituitary and adrenal glands, the gallbladder, the fallopian tube and the uterus and in renal as well as in bronchial tumors.[6]

Since the onkocytes are always the products of a transformation of the parenchymal cells of the respective organs, they represent a peculiar degeneration. This transformation appears permanent in view of the increase of onkocytes with age in the most thoroughly examined organs. Finally, cells of various organs which were previously very different become morphologically similar, even if they still resemble in shape and arrangement the cells from which they stem.

The onkocytes of various organs are known under different names; e. g., those of the parathyroid glands are known as oxyphilic cells of Welsh.

A special discussion is required for these cells in the thyroid glands, since different classifications have created confusion. Besides the main cells which form the bulk of the epithelium, the following types of cells can be differentiated:

1. Onkocytes. These reveal the tinctorial characteristics of this type of cell, especially the metachromasia on inclusion staining. They are relatively large cells in line with the alveolar epithelium. They seem to have been derived by a peculiar degeneration from the latter. They are easily recognized in routine hematoxylin-eosin stains because of the frankly red and uniformly fine granular cytoplasm. Their incidence increases with age. They are especially common in exophthalmic goiter in which they sometimes comprise entire lobules.[7]

2. Colloid cells. Langendorff's [8] colloid cells have a homogeneous cytoplasm which, like colloid, is strongly stained. They are, for the most part, smaller than the main cells. According to Bastenie,[9] Barg-

6. Hamperl, H.: Virchows Arch. f. path. Anat. **296**:327, 1936.

7. Zippel, L.: Virchows Arch. f. path. Anat. **308**:360, 1941.

8. Langendorff, O.: Arch. f. Anat. u. Physiol. (supp.) 1889, p. 1.

9. Bastenie, P.: Ann. et bull. Soc. roy. d. sc. méd. et nat., de Bruxelles **71**:72, 1935.

mann [10] and others, they appear to be exhausted, if not necrobiotic, main cells. They are seen in all ages, even in the fetus.

3. Interfollicular (parafollicular) cells. These were first described by Baber [11] as parenchymatous cells and then by Hürthle [12] as "large cells rich in cytoplasm"; Nonidez [13] demonstrated granules in their cytoplasm with silver impregnation and gave them the aforementioned name. Nevertheless, they are often called Hürthle cells without sufficient justification. They were mainly described in the thyroid gland of the dog by the authors mentioned previously. The number of the granules and their affinity for silver vary with the age of the dog. The interfollicular cells of the dog are different from those of the rat or the rabbit [14] as far as shape, size and impregnation of the granules are concerned.

It is generally assumed that the interfollicular cells of Nonidez occur also in the human thyroid gland.[15] However, it cannot be expected that these cells should appear identical with those of the dog. Elements analogous to the interfollicular cells of Nonidez occur in man in line with the follicular epithelium. These cells are very large, have large nuclei and a light, agranular cytoplasm (*"helle Zellen* of Feyrter" [16]). Similarly to the cells of the dog, they reveal a tendency to extend into the stroma and to leave the line of follicular epithelium. However, it is still problematic whether all interstitial cells of the human thyroid gland correspond to the interfollicular cells of Nonidez. Some may also be main cells budding during the course of new formation of follicles. Others may be tangential cuts of follicles or may even be connective tissue cells. In the human thyroid gland the large light cells of the follicular epithelium and all interstitial cells (whether or not they belong to the interfollicular cells of Nonidez) as well as the colloid cells are easily differentiated from the typical onkocytes. The latter are in line with the follicular epithelium, have a densely granular cytoplasm and are metachromatic. Nevertheless, in the past there has been confusion because the characteristics of the onkocytes were not known.

As in the normal thyroid gland, so in thyroid tumors the epithelial cells may become onkocytes. If the onkocytes of the normal thyroid

10. Bargmann, W., Innersekretorische Drüsen, in v. Moellendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1939, vol. 6, p. 11.

11. Baber, E. C.: Phil. Tr. Roy. Soc., London **166**:557, 1877; **172**:572, 1882.

12. Hürthle, K.: Arch. f. d. ges. Physiol. **56**:1, 1894.

13. Nonidez, J. F.: Am. J. Anat. **49**:479, 1932; **53**:339, 1932; **56**:131, 1933.

14. Raymond, N.: Anat. Rec. **53**:355, 1932.

15. Zechel, G.: Anat. Rec. **56**:119, 1933. Altmann, H. W.: Beitr. z. path. Anat. u. z. allg. Path. **104**:421, 1940. Bakay, L., Jr.: Virchows Arch. f. path. Anat. **314**:329, 1947.

16. Feyrter, F.: Wien. Ztschr. f. inn. Med. **27**:9, 1946.

gland are erroneously considered as Langendorff's colloid cells or parafollicular cells, the same error may occur with tumors.

Langhans [17] already has recognized a form of large cell, microfollicular thyroid adenoma. Zippel [7] and Hamperl [8] found a transition between thyroid adenoma containing scattered onkocytes and the adenoma entirely composed of them. The latter tumors correspond to the large cell, microfollicular adenoma of Langhans. Microfollicular adenomas or cysts in the adenoma may even be lined by onkocytes.[6] The onkocytic transformation of thyroid epithelium may therefore occur in every type of adenoma, while the arrangement of the tumors may be preserved. This transformation can be seen even in carcinoma, as is evidenced by a case of Langendorff previously reviewed.[6]

Getzowa [18] referred to the similarity of the large eosinophilic cells in thyroid tumors and the Welsh cells of the parathyroid glands. Obviously this is based on the fact that both the large cells of the thyroid tumors and the oxyphilic cells of Welsh of the parathyroid glands are organ cells transformed to onkocytes. Getzowa expressed the belief that the large cells in thyroid tumors prove that these tumors evolve from the parathyroid gland, an opinion held until very recently.[19] However, several investigators, among them, Willis,[20] emphasized that "large-eosinophilic-celled adenomas and carcinomas almost certainly arise from ordinary thyroid epithelium."

The onkocytes of the thyroid gland and thyroid tumors were also confused with the interfollicular cells of Hürthle. Ewing [21] examined two tumors answering this description (large-cellular, microfollicular adenoma of Langhans) and suggesting that the acidophile cells may represent hypertrophic cells of the thyroid alveoli." He illustrated a tumor obviously composed of onkocytes as "encapsulated acidophile cell adenocarcinoma, Hürthle cell tumor" (his fig. 492 a). Subsequently, such tumors were designated in the American literature as Hürthle cell tumor.[22] Willis [20] rightly said, "There is no real evidence that the tumors are related to the cells described by Hürthle." The nature of this tumor has been well understood by Wilensky and Kaufmann [23] and others,[24] who considered the appearance of the large eosinophilic cells as

17. Langhans, T.: Virchows Arch. f. path. Anat. **189**:69, 1907.
18. Getzowa, S.: Virchows Arch. f. path. Anat. **188**:181, 1907.
19. Eisenberg, A. A., and Wallenstein, H.: Arch. Path. **13**:716, 1932.
20. Willis, R. A.: Pathology of Tumors, London, Butterworth & Company, Ltd., 1948.
21. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders & Company, 1934.
22. Haagensen, C. D.: Am. J. Cancer **15**:2044, 1931.
23. Wilensky, A. O., and Kaufmann, P. A.: Surg., Gynec. & Obst. **66**:1, 1938.
24. Clute, H. M., and Warren, S.: Surg., Gynec. & Obst. **60**:861, 1935. Morrow, W.: Arch. Path. **40**:387, 1945.

a "degenerative change" and thought that "this conversion is a secondary change which may involve the entire tumor." [23] Since this degeneration and conversion do not lead to the colloid cells of Langendorff or the parafollicular cell of Hürthle, but to onkocytes, it would be better to discard the term "Hürthle cell tumor" and to speak of thyroid adenoma or carcinoma with partial or complete onkocytic transformation (degeneration).

Recently, Bakay [25] described parafollicular cell adenoma of the thyroid gland. This would be a tumor composed of the Baber-Hürthle-Nonidez cells. Unfortunately, the histologic evidence for this designation is not convincing; I feel rather that these tumors represent trabecular thyroid adenoma, the cells of which are partially transformed to onkocytes.

SUMMARY

Onkocytes are easily demonstrated by inclusion staining with thionine and cresyl violet (Feyrter [4]). They occur not only in the salivary glands but also in other organs, e. g., the parathyroid glands (oxyphilic cells of Welsh) and the thyroid gland. In the thyroid gland they have been confused with the colloid cells of Langendorff and the parafollicular cells of Hürthle. The onkocytic transformation of thyroid epithelium occurs also in thyroid adenoma and carcinoma. This has resulted in the incorrect designation of the large-cellular microfollicular adenoma of Langhans as Hürthle cell tumor. Actually, it represents adenoma or carcinoma in which the epithelial cells have been more or less completely transformed to onkocytes. The term "Hürthle cell tumor" should therefore be abandoned.

25. Bakay, L., Jr.: Arch. Path. **45**:447, 1948.

NATURE OF THE LIPID OF HYALINE ARTERIOLOSCLEROSIS

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ARTERIOLOSCLEROSIS of the kidney apparently bears a relationship to hypertension. Some believe that the arteriolosclerosis is the first change and a cause of hypertension. Some believe that the hypertension is the first change and a cause of arteriolosclerosis. Others suggest that arteriolosclerosis and hypertension are independent processes. However, arteriolosclerosis of the kidney occurs so frequently with hypertensive vascular disease that it is surprising when this interesting arteriolar change is not present. One expects to find it in sections of kidneys from those who have had hypertensive cardiovascular disease during life. Whether arteriolosclerosis is the cause of hypertension, the result, or unrelated, more should be known about it. For example, is it the same disease as atherosclerosis of the larger vessels?

Arteriolosclerosis has been described in at least three forms.[1] The first is intimal hyalinization, which is the most conspicuous, because the hyaline material stains well with eosin. Intimal fibrosis is a second form, and, third, there may be changes in the media. The hyaline material has greatest interest because it is a region of lipid substance, like the atheroma of larger vessels. We have been studying the lipid in hyaline arteriolosclerosis by two methods, by staining it and by testing its solubility.

The ability of the lipid of the hyaline arteriole to absorb scarlet red was mentioned by Prym[2] in 1904 and by Jores[3] in 1905. A plate accompanying Prym's article illustrates the scarlet red ("Scharlach R") staining of hyaline arterioles of kidney and pancreas.

"The chemical composition of the hyaline substance has not been determined," wrote Bell and Clawson[4] in 1928, and they continued:

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1. Moritz, A. R., and Oldt, M. R.: Am. J. Path. **13**:679, 1937.
2. Prym, P.: Virchows Arch. f. path. Anat. **177**:485, 1904.
3. Jores, L.: Virchows Arch. f. path. Anat. **178**:367, 1905.
4. Bell, E. T., and Clawson, B. J.: Arch. Path. **5**:939, 1928.

"Hueck considers it a combination of various coagulated bodies. Some investigators believe that it is filtered through the endothelium into the wall of the vessel. Often it is possible to demonstrate fatty material in the hyaline material."

OBSERVATIONS ON STAINING

In ordinary paraffin sections the hyalin stains diffusely with eosin, like hyalin in many conditions, as in amyloid deposits, fibrinoid changes and necrotic muscle fibers. The hyaline appearance in paraffin sections is not due to the lipid material, because the fat solvents used in preparing the sections have removed it. This we have demonstrated by failing to stain the hyalin in paraffin sections with sudan IV. What we see in paraffin sections is perhaps carbohydrate or protein, with which we are not at present concerned.

In microscopic sections prepared by freezing and then cutting, free of contact with fat solvents, the lipid is still present. It absorbs sudan IV dye, also known as scarlet red or Scharlach R, and occurs in the same region which stains with eosin in the paraffin section. Sudan IV stains diffusely or in a granular or vacuolated form. This ability of lipid to absorb the sudan dyes is called sudanophilia. Sudan IV is made up in a mixture of alcohol and acetone. Because the dye is more soluble in the lipid of the hyaline vessel than in these solvents, the lipid by a purely physical staining process takes up sudan IV from the solvents.

A comment is needed here concerning definitions. According to Bloor,[5] lipids may be defined as a group of naturally occurring substances comprising the higher fatty acids, their naturally occurring compounds and substances found naturally in chemical association with them. The group is characterized in general by insolubility in water and solubility in so-called fat solvents, e. g., ether, chloroform and benzene. The term "fats" has been used, especially in the past, to designate the whole group, but in Bloor's classification fats are simple lipids, just one category of lipids, and the term "lipoid" is not used by him. We shall try to keep to Bloor's classification, though it is easy to slip back into the use of "fats" to cover the whole group.

Other oil-soluble dyes occur in addition to sudan IV, such as sudan III, oil red O and sudan black. The ability of the lipid to absorb any of these dyes is spoken of as sudanophilia. Thus oil red O showed sudanophilia of marked degree in the hyaline renal arterioles of a Negro man of 33 years who died with hypertensive cardiovascular disease. Sudan black demonstrated sudanophilia of lipid along the course of an arteriole in another case. The lipid was discontinuous in distribution.

5. Bloor, W. R.: Biochemistry of the Fatty Acids, New York, Reinhold Publishing Corporation, 1943.

What is the significance of the staining of a substance with the oil-soluble dyes? It appears to indicate that the substance is a lipid but does not designate what sort of lipid precisely.[6] Moreover, tissues which are known by chemical extraction to have lipids in them may exhibit no sudanophilia.

Frozen sections of the atherosclerotic aorta, for comparison, show sudanophilia of intimal tissue and of macrophages. Human adipose tissue shows sudanophilia of a high degree.

Other stains point in the direction of certain substances, but we have not done enough tests to express generalities or interpretations. In preliminary studies the Schultz modification of the Liebermann-Burchardt method[7] for sterols does give the characteristic greenish reaction, suggesting a cholesterol content, but it is most difficult to identify the arterioles conclusively in the frozen sections.

TABLE 1.—*Degree of Sudanophilia of Lipid of Hyaline Arteriolosclerosis in Frozen Sections of Unfixed Human Kidney Tissue After Action of Solvents*

	1 min.	2 min.	4 min.	8 min.	19 hr.	24 hr.	36 hr.
Petroleum benzin.....	43?2?	44?7?	43?3?	3?3	44	3?2?23?27?3?	?1
Acetone.....	0000	0000	0000	000	00	000000000000	00
Benzene.....	3?3?3	44?3?	44?3?	4?3	3?	1000?12?30?30	??
Chloroform.....	4?3?3	2?3?3	34?3?	?41	??	000?2?00?000	?
Absolute alcohol.....	0000	0000?	0000	000	00	0000000000	00

Key to tables 1 to 5: The degree of sudanophilia is estimated from 0 to 4, with 4 indicating the degree of staining of lipid that had not been treated with any solvent. A question mark indicates that the frozen section was technically unsatisfactory. The solvents were at room temperature.

The Marchi method, using osmium tetroxide (commonly called osmic acid) after mordanting with potassium bichromate, is usually positive.

SOLUBILITY OF THE LIPID

To study the solubility of the lipid material in the usual fat solvents, frozen sections were cut and placed in the solvents at room temperature for varying intervals, and then stained with sudan IV to determine how much of the sudanophilic material had been removed. The degree of staining with sudan IV was indicated by grades of 0 to 4. Grade 4 staining was that observed in the control, which had not been treated with solvent. Table 1 indicates that the lipid of the hyaline arterioles in unfixed kidney is extracted by acetone or absolute alcohol in as short a time as one minute. Extraction by petroleum benzin, benzene and chloroform did not occur in a matter of minutes, and extraction by

6. Lee, B.: The Microtomist's Vade-Mecum, ed. 10, Philadelphia, P. Blakiston's Son & Company, Inc., 1937.

7. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

petroleum benzin did not occur even after a day. Benzene and chloroform extractions, however, were nearly complete after a day. Table 2 indicates the same tendencies in formaldehyde-fixed kidney. Acetone and absolute alcohol do not extract the lipid so completely, in a matter of minutes, from the fixed as from the unfixed kidney. At the end of a day the extraction following acetone, absolute alcohol and chloroform is frequently complete, but petroleum benzin extraction, again, did not occur even after a day.

TABLE 2.—*Degree of Sudanophilia of Lipid of Hyaline Arteriolosclerosis in Frozen Sections of Formaldehyde-Fixed Human Kidney Tissue After Action of Solvents*

	1 min.	2 min.	4 min.	8 min.	19 hr.	21 hr.	24 hr.	29 hr.	36 hr.
Petroleum benzin	4344	1434	3444	234	4	722	3	42	43
Acetone	0001	1111	1001	001	0	110	0	00	00
Benzene	3244	1243	3333	213	1	121	0	21	11
Chloroform	4144	2220	4443	122	0	011	0	01	00
Absolute alcohol	2010	2120	1122	103	0	111	1	00	00

TABLE 3.—*Degree of Sudanophilia of the Diffuse Lipid of Formaldehyde-Fixed Human Aortic Atheroma After Action of Solvents*

	1 min.	2 min.	4 min.	8 min.	16 hr.	24 hr.	48 hr.
Petroleum benzin	4333	4344	4443	4343	110	00	121
Acetone	0100	0000	0000	0101	000	00	000
Benzene	3427	4051	3434	3302	000	00	010
Chloroform	2322	2411	2121	4101	000	00	000
Absolute alcohol	1113	0042	0000	0011	000	00	000

TABLE 4.—*Degree of Sudanophilia of Macrophages of Formaldehyde-Fixed Human Aortic Atheroma After Action of Solvents*

	1 min.	2 min.	4 min.	8 min.	16 hr.	24 hr.	48 hr.
Petroleum benzin	4443	4444	4443	4444	332	21	202
Acetone	2211	2211	2211	2211	121	22	111
Benzene	3444	3444	4444	3432	221	21	011
Chloroform	4444	2444	4344	4333	001	11	000
Absolute alcohol	1313	2224	2311	2212	120	10	111

Control fixed tissue placed in isotonic solution of sodium chloride for twenty-four hours showed 4 plus staining of the lipid of hyaline arteriolosclerosis, indicating that the material is not water soluble.

Tables 3 and 4 show the effect of the solvents on the lipid of formaldehyde-fixed aortic atheroma, while table 5 presents the results on formaldehyde-fixed adipose tissue.

From table 3 it is noted that the diffuse lipid (not in macrophages) of the aortic atheroma was quickly removed by acetone and usually, but not always, by absolute alcohol, but not by petroleum benzin, benzene

or chloroform. This was similar to the behavior of the lipid of hyaline arteriolosclerosis. At the end of a day the lipid of the atheroma was removed by all the solvents except petroleum benzin, and the removal after this interval was a trifle more complete than from the hyaline arteriole.

Table 4 indicates that the macrophages are more tenacious of the lipid, removal by acetone and absolute alcohol not occurring in the first few minutes.

Table 5 shows that acetone removes the lipid of adipose tissue in a few minutes, but that absolute alcohol is not as effective as it is with the lipid of hyaline arteriolosclerosis. Petroleum benzin again fails to extract as well as the other solvents.

COMMENT

The studies of solubility indicate that there is lipid in the region of the arteriolar hyaline change, for the ordinary fat solvents extract most

TABLE 5.—*Degree of Sudanophilia of Formaldehyde-Fixed Human Adipose Tissue After Action of Solvents*

	1 min.	2 min.	4 min.	8 min.	15 hr.	30 hr.	24 hr.	48 hr.
Petroleum benzin.....	3444?	3443	3343	443	434	44	04402	43
Acetone.....	4000000	0002	0000	000	0	00	00000	00
Benzene.....	44443	1443	3443	243	22	00	2000	00
Chloroform.....	42341	2944	3421	222	100	00	00000	00
Absolute alcohol.....	31444	3132	4124	124	000	0	000000	00

of the lipid in twenty-four hours. Acetone and absolute alcohol remove the material readily and quickly. The failure of petroleum benzin, benzene and chloroform to remove the material as readily may be a matter of slower penetration of the tissue. Acetone and absolute alcohol are miscible with water, while the other three solvents are not. It seems probable that acetone and absolute alcohol are able to penetrate the watery medium of the frozen section quickly and thus remove the lipid. It is also possible that the lipid is of a particular sort on the basis of the solubilities indicated, and in this connection the readings at the end of twenty-four hours are probably of greater significance than those after a few minutes.

After consultation with chemists, some of whom have had investigative experience in the field of lipid chemistry, we are unable to identify the lipid specifically or to conclude more than that the substance is a lipid.

The similarities of arteriolosclerosis and arteriosclerosis are evident. The lipids of both processes stain with the oil-soluble or sudanophilic dyes, and the solubilities of the lipid of the hyaline arteriole are similar to those of the diffuse lipid of the atheroma. In both instances

the lipid may represent an imbibition of lipid by an altered or damaged vessel wall. The arteriolar lipid usually exhibits no tendency to be phagocytosed as does the atheromatous lipid, nor does it usually exhibit calcification. These secondary changes, however, may be absent from arteriolar lipid because it occurs in such comparatively small masses.

SUMMARY

The hyaline material of renal arteriolosclerosis is in part lipid and in part nonlipid. The nonlipid part stains well in ordinary paraffin sections which have passed through fat solvents in the process of preparation, and from which the lipid has been extracted.

The lipid part (or at least the nonbound lipid) of hyaline renal arteriolosclerosis absorbed the sudanophilic dyes readily, such as sudan IV, sudan black and oil red O, when studied in unfixed or formaldehyde-fixed frozen sections.

In preliminary studies the Schultz modification of the Liebermann-Burchardt method for sterols gave the characteristic greenish reaction, suggesting a considerable cholesterol content of the hyaline regions of renal arteriolosclerosis; the Marchi method, using osmium tetroxide after mordanting with potassium bichromate, was usually positive for unsaturated fatty acids.

Solubility tests of the lipid of arteriolosclerosis were made by immersing frozen sections of kidney in various fat solvents (petroleum benzin, acetone, benzene, chloroform and absolute alcohol) for varying intervals of time and then staining with sudan IV to determine the amount of sudanophilic material which had been extracted.

These tests indicated that the sudanophilic lipid was dissolved from unfixed frozen sections by acetone or absolute alcohol at room temperature in as short a time as one minute, while it was not dissolved by petroleum benzin at the end of twenty-four hours. Benzene and chloroform often dissolved the lipid completely in twenty-four hours. The rapid solvent action of acetone and absolute alcohol was attributed to their property of miscibility with the aqueous components of the tissues.

Formaldehyde-fixed hyaline arterioles of kidney were a trifle more tenacious of the lipid than the unfixed arterioles when treated with solvents.

The diffuse lipid of atheromas of the aorta had staining reactions and solubilities similar to those of the lipid of arteriolosclerosis. The lipid contained in macrophages of atheromas was less readily dissolved by fat solvents than the diffuse lipid.

The staining reactions and solubilities point to similarities between arteriolosclerosis and atheromatosis of the aorta.

NEUROTROPIC AND VISCEROTROPIC STRAINS OF COLUMBIA SK AND ENCEPHALOMYOCARDITIS VIRUS

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THE EXTENT to which various murine strains of poliomyelitis or poliomyelitis-like viruses are capable of inducing in experimentally infected animals central nervous system lesions or peripheral lesions in the skeletal or the heart muscle, or both, has become a subject of increasing interest in recent years. Neuromuscular lesions in the paralyzed limb have been described with simian as well as with murine virus. They have either taken the form of regional obliteration of the motor end plate [1] or have appeared as a localized but intense myositis with the presence of an inflammatory exudate and total necrosis of individual muscle fibers.[2] Pathologic changes occurring in the heart muscle of paralyzed animals have been defined essentially as a myocarditis characterized by varying degrees of interstitial inflammation and occasional areas of muscle cell degeneration.[3] The aforementioned experimental observations are supplemented by the fact that structural and functional changes at the motor end plate, as well as myocarditis, have also been encountered in fatal and nonfatal human cases of the disease.[4] When the problem is further examined from the point of

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view of studying the growth requirements of the virus under controlled conditions, it is found that certain strains of murine poliomyelitis virus, including the Lansing strain, will readily multiply in vivo or in vitro on extraneural tissues.[6]

The evidence collected by clinical and laboratory workers therefore seems to converge to a point where it becomes of interest to raise the question whether the agent or agents which cause poliomyelitis in man —as the term is commonly understood on clinical, pathologic and epidemiologic grounds—may not possess both neurotropic and viscerotropic properties. The present paper deals with an experimental study of this problem in which Columbia SK and encephalomyocarditis virus were used as test agents. These two viruses are especially suitable for this purpose because of their unusually high invasiveness and systemic distribution in the infected organism. While they are, at present, not definitely classified, mostly because of serologic aberration, they bear a close relationship to the poliomyelitis group, as indicated by the fact that both viruses are capable of paralyzing cynomolgus monkeys with typical poliomyelitic lesions of the cord.[6]

EXPERIMENTAL WORK

Experiments with Columbia SK Virus.—When this virus is harvested from the brains of paralyzed mice or guinea pigs and is passed by intracerebral or by intraperitoneal injection from mouse to mouse or from guinea pig to guinea pig, as the case may be, the paralyzed animals present the familiar symptoms and lesions of encephalomyelitis of mice and of poliomyelitis of guinea pigs, as previously described by ourselves[7] and by Wolf.[8] Animals thus infected show no evidence of any local lesions in the affected skeletal muscle or in the heart muscle. However, when the same virus is introduced directly into the skeletal muscle an intense local myositis is produced in the injected paralyzed limb, as was first demonstrated by Rustigian and Pappenheimer.[2a] The same phenomenon may readily be obtained in intramuscularly infected guinea pigs except that development of the lesion requires more time, in keeping with the fact that in guinea pigs the virus is of lower virulence

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and the experimental disease of slower course than in mice. An illustration of the characteristic type of lesion observed in the paralyzed leg of a guinea pig six days after the virus has been intramuscularly introduced into the same limb is given in figure 1.

Because in paralyzed mice the virus is found to have wide extraneural distribution, a finding which is constant irrespective of the chosen route of infection, we decided to collect virus from the spleen of an intracerebrally infected mouse (four hundred and fifteenth serial passage) and to attempt carrying the strain by intraperitoneal injection from mouse to mouse, using freshly ground infected spleen in dilutions of 10^{-2} and 10^{-3} as inoculum for the next passage.[9] In ten consecutive passages it was thus possible, with some difficulty, to establish a "visceral" strain which still caused fatal paralysis in mice but had undergone an obvious deterioration in potency (10^{-5} intracerebral; 10^{-3} intraperitoneal) as compared with initial

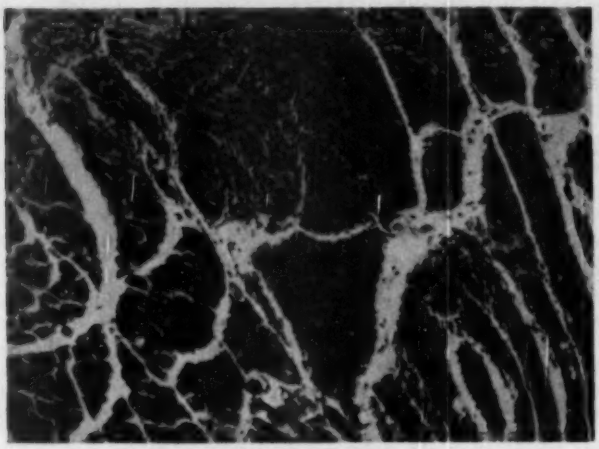

Fig. 1.—Paralyzed leg muscle of guinea pig infected intramuscularly with the brain strain of Columbia SK virus, showing myositis with extensive destruction of muscle fibers.

splenic virus, from the first passage (10^{-5} intracerebral; 10^{-3} intraperitoneal). At the same time a "neural" strain was carried over ten parallel consecutive passages by injecting it into mice intracerebrally, routine dilutions of 10^{-7} and 10^{-8} prepared from freshly harvested brain being used.

After the tenth serial passage the visceral strain was used to infect mice intraperitoneally. After paralysis set in, the animals were killed and examined histologically for peripheral lesions. It was found that such mice showed regularly a localized myositis in the paralyzed limbs and a focal myocarditis. No comparable lesions could be detected in the skeletal muscle or the heart muscle of mice paralyzed by intraperitoneal injection of the tenth passage neural strain. Examples of these lesions are illustrated in figure 2.

9. The mice used in these and subsequent experiments were albino Swiss mice of the Rockland strain and weighed between 8 and 12 Gm.

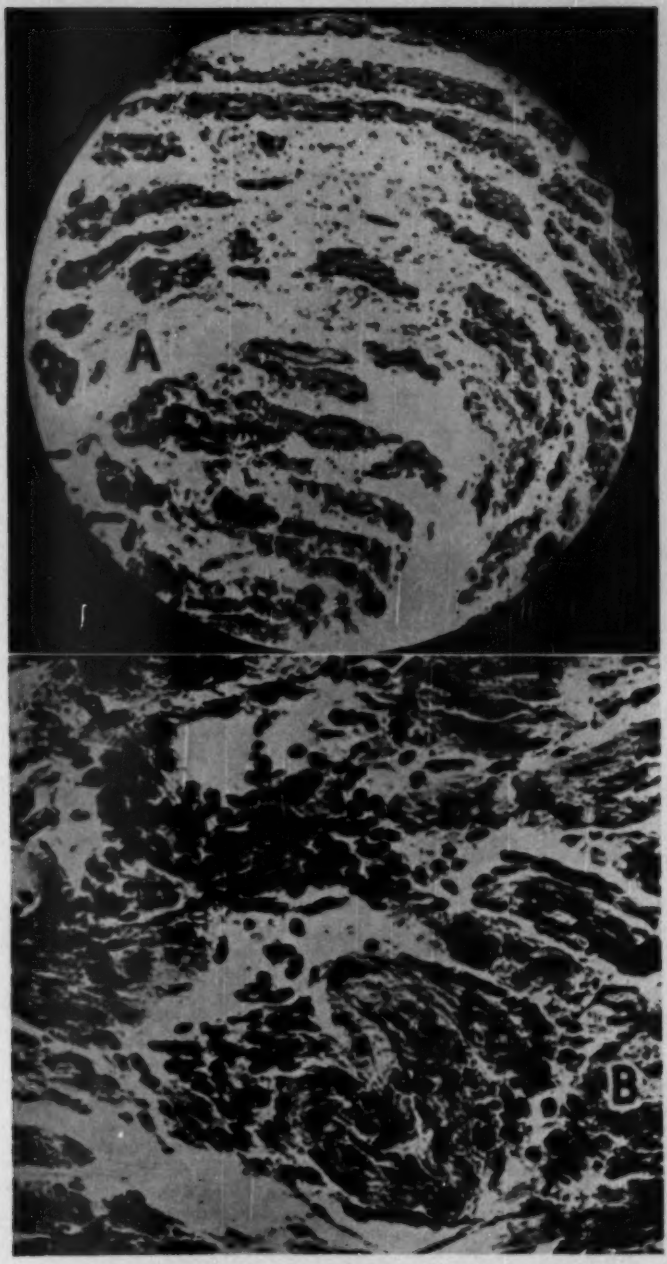

Fig. 2.—*A*, paralyzed leg muscle of mouse infected intraperitoneally with splenic strain of Columbia SK virus, showing diffuse myositis with muscle cell necrosis. *B*, heart muscle of mouse paralyzed by intraperitoneal injection of splenic strain of Columbia SK virus, showing focal myocarditis.

Experiments with Encephalomyocarditis Virus.—The virus was received from Dr. J. Warren (Army Medical School, Washington, D. C.) in its fifty-second serial mouse passage. Mice infected in this laboratory after subsequent mouse passages by either the intracerebral or the intraperitoneal route showed typical encephalomyelitis with extensive lesions of the central nervous system, but careful histologic examination of the heart or paralyzed skeletal muscle failed to reveal any significant changes.

Attempts to develop a visceral strain of the virus were made by using the same technic as previously described for Columbia SK virus. After ten consecutive passages of infected spleen such a strain could readily be established. The splenic strain still paralyzed mice but showed a drop in virulence similar to that observed with Columbia SK virus. A conventional neural strain was carried in mice at the same time by injecting intracerebrally virus harvested from brain.

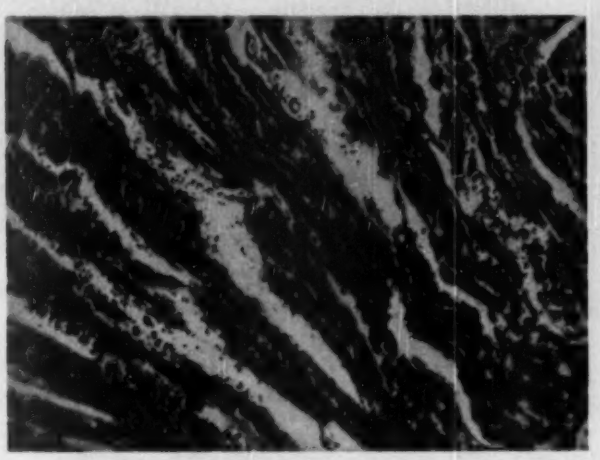

Fig. 3.—Heart muscle of mouse paralyzed by intraperitoneal injection of splenic strain of encephalomyocarditis virus, showing extensive interstitial myocarditis.

When mice infected intraperitoneally with the visceral strain (tenth passage) were examined histologically, it was found that the paralyzed animals presented regularly myocardial lesions and extensive destruction of muscle fibers in the affected skeletal muscle. These lesions are portrayed in figures 3, 4 and 5. No such lesions were found in the mice paralyzed by the neural strain.

Immunologic Tests.—The obvious alteration of tissue tropism which had occurred with both viruses (Columbia SK and encephalomyocarditis) as the result of passing peripheral viral material by a peripheral route prompted us to determine whether this change in biologic properties was possibly associated with changes in antigenic properties. Cross neutralization tests were therefore set up with the two viruses, to determine in each case whether the visceral and the neural strain were inactivated by antiserums prepared by immunizing rabbits with either brain virus or splenic virus. Suffice it to say that in each case the splenic virus antiserum was only moderately effective against the splenic virus and possessed little or no

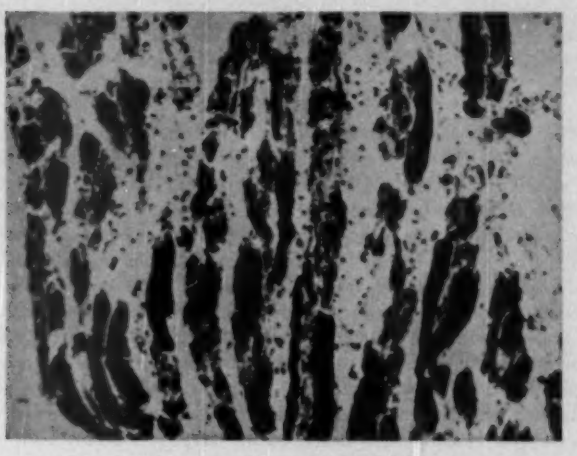

Fig. 4.—Paralyzed leg muscle of mouse infected intraperitoneally with splenic strain of encephalomyocarditis virus, showing myositis with muscle cell necrosis.

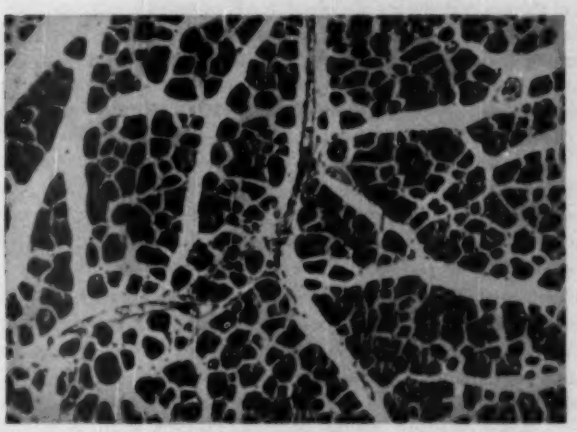

Fig. 5.—Nonparalyzed leg muscle of mouse infected intraperitoneally with splenic strain of encephalomyocarditis virus, showing normal muscle. Same mouse as in figure 4.

neutralizing capacity against the brain virus, whereas the brain virus antiserum inactivated brain and splenic virus equally well. The observed differences are more readily explained on a quantitative than on a qualitative basis. In other words, it would seem that the splenic virus, owing to its depressed virulence, was a poorer antibody-producing agent rather than that the brain virus functioned as a broader antigen.

COMMENT

The observations made in this paper indicate that multiplication of Columbia SK and of encephalomyocarditis virus on non-neural tissue, i. e., spleen, is followed by a definite depression in virulence. This loss of virulence may be demonstrated as a marked reduction of titer following intracerebral or peripheral injection of the virus, without any significant prolongation of the incubation period or the length of survival after paralysis. Associated with this change is a restoration or a preservation of the ability of these agents to induce characteristic lesions in the heart and skeletal muscle of intraperitoneally infected mice, a property which apparently is temporarily lost or remains dormant when the virus is propagated on brain during intracerebral passage. We have been unable to discover well defined myocardial lesions in our stock of normal mice, such as have recently been described by Gray.[10] Possibly such spontaneous lesions are caused by latent Theiler's virus infection, which is known to occur to various degrees in different colonies of mice.

The myositis produced in the paralyzed limb by intraperitoneal injection of the visceral strains of Columbia SK or encephalomyocarditis virus places these viruses in close biologic relationship with the viruses belonging to the Coxsackie group.[10a] In fact, a remarkable grading of the intensity of this "myotropism" among various strains of murine poliomyelitis or poliomyelitis-like viruses is clearly recognized as one traces the development of this property from its rudimentary inception with the neural strains of Columbia SK or encephalomyocarditis virus, which produce myositis only on being introduced directly into the muscle, to its fully perfected state with the visceral strains of these viruses or the Coxsackie viruses, all of which are capable of producing the same pathologic effect by extramuscular routes of infection.

Fundamentally similar changes occurring in tissue tropism under experimental conditions have been reported for other viruses. Examples are, on the one hand, the gain of neurotropism and the loss of viscerotropism resulting from intracerebral passage of the yellow fever virus in mice [11]; on the other, the loss of neurotropism and the gain of dermotropism when Western equine encephalomyelitis virus is transmitted

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11. Theiler, M.: Ann. Trop. Med. **24**:249, 1930.

serially in the pads of guinea pigs.[12] The phenomenon described in this paper suggests that viruses belonging to the poliomyelitis group may possess similar facilities for exchange of tissue affinities, without undergoing any marked alteration in antigenic composition.

SUMMARY

When mice are infected intraperitoneally with Columbia SK or encephalomyocarditis virus which has been harvested from brain and passed as a brain strain intracerebrally, the paralyzed animals show lesions of the central nervous system but lesions of the skeletal or the heart muscle are not demonstrable.

When mice are infected intraperitoneally with Columbia SK or encephalomyocarditis virus which has been harvested from spleen and carried as a splenic strain peripherally over ten serial passages, paralysis is associated with myositis of the affected skeletal muscle and with myocarditis.

The experimental data suggest that murine viruses belonging to the poliomyelitis group may possess both neurotropic and viscerotropic properties.

12. Olitsky, P. K.; Cox, H. R., and Syverton, J. T.: J. Exper. Med. **59**:159, 1934.

HEMORRHAGIC ENCEPHALOPATHY DUE TO DISSEMINATED THROMBOCYTIC THROMBOSIS

Report of a Case

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AND
R. S. LEE, B.Sc.
ST. LOUIS

HEMORRHAGIC ENCEPHALOPATHY has been produced by a variety of etiologic agents, such as heavy metals,[1] widely disseminated embolic fat fragments,[2] vitamin deficiency [3] and trauma.[4] Petechial lesions scattered throughout the brain substance have been encountered in hemorrhagic encephalitis of viral origin.[5] A few cases of disseminated visceral vasothrombosis recorded in the literature [6] have presented a baffling neurologic disorder with a lethal termination. A similar case, in which the clinical picture was suggestive of encephalitis and "diffuse brain purpura" was observed, attributed to disseminated thrombocytic thrombosis occurring within arterioles and capillaries, is reported.

REPORT OF CASE

D. P., a 53 year old white man, was admitted to the hospital four days before death, complaining of pronounced vertigo and rapidly increasing mental confusion of three days' duration. Examination revealed a pale, mildly jaundiced patient, acutely ill and extremely lethargic. The patient's response to questioning was notably sluggish and incoherent. Past and family histories were not pertinent. On admission widespread ecchymoses and petechiae were noted over the abdomen and the arms, and, at the same time, the initial neurologic examination revealed marked weakness of all four limbs. During hospitalization it was noted that the plantar responses at first were equivocal; later a dominant extensor response developed in the lower limbs. The blood pressure on admission was 136 systolic and

From the Department of Pathology, St. Louis University School of Medicine.

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6. (a) Fitzgerald, P. J.; Auerbach, O., and Frame, E.: Blood **2**:519, 1947. (*b*) Engel, C. L.; Scheinker, I. M., and Humphrey, D. C.: Ann. Int. Med. **26**:919, 1947. (*c*) Muirhead, E. E.; Grass, G., and Hill, J. M.: Am. J. Clin. Path. **18**:523, 1948.

80 diastolic, and ophthalmoscopic examination revealed choking of the optic disks. The patient had a constantly elevated temperature of 101 to 103 F. and a persistent white cell count of around 15,000 per cubic millimeter. The differential count was typical: metamyelocytes, 15 per cent; band cells, 14 per cent; segmented cells, 58 per cent; eosinophilic myelocytes, 7 per cent; lymphocytes, 25 per cent, and monocytes, 5 per cent. Few thrombocytes were noted in smears. Further laboratory analyses revealed severe anemia, the blood count showing 2,200,000 erythrocytes and 8 Gm. of hemoglobin; a serum test for syphilis was negative, and blood cultures on three successive days were sterile. There was rapid deterioration of the patient's condition, with lethargy and irrationality passing rapidly into semicoma. A lumbar puncture on the day before death showed a clear fluid at 280 mm. of water pressure, with the total protein 94 Gm., chlorides 775 mg., sugar 80 mg. and a cell count of 5 erythrocytes and leukocytes. The Wassermann reaction was negative, and the Lange colloidal gold reading was 0000000000. The patient lapsed into a profound coma, Cheyne-Stokes respirations followed, and death occurred seven days after the onset of this fatal illness. The clinical diagnosis was cerebral thrombosis, cause and site undetermined.

Necropsy.—Gross observations: The body was that of a well developed man with marked pallor and jaundice. Scattered over the surface of the abdominal wall and the lower extremities were numerous ecchymoses. No palpable lymph nodes were noted. Visceral examination showed purpuric blotches in the spleen and the left kidney. The brain weighed 1,465 Gm.; it was bulging and tight against the meninges, with a moist cut surface. Throughout the brain substance, particularly in the subcortical and centrum ovale regions, there were widespread petechial hemorrhages, both hemispheres being equally involved. These petechiae measured up to 0.3 cm. in diameter. The remainder of the organs were free of pertinent macroscopic alterations.

Histopathologic Observations: Many of the small vessels of the heart contained hyaline thrombi, formed by the agglutination of thrombocytes. The alveoli of the lungs contained a serous exudate with many hemosiderin-laden macrophages; within the capillaries there were only a few hyaline thrombi. In the liver there were small focal areas of necrosis, with neutrophilic leukocytes. There were many focalized regions of hemorrhage in the pulp of the spleen, and many of the arterioles showed hyaline thickening. The capillaries of the pancreas revealed occlusion by hyaline thrombi with associated foci of necrobiosis. In the kidney, proliferation of glomerular endothelium was noted, and a few pink thrombotic masses within the glomerular tufts (fig. 1). A few megakaryocytes were incarcerated in the glomerular capillaries. Intratubular hemoglobin casts, pronounced hyaline droplet formation and fatty degeneration of the tubular epithelium were evident. Many focal areas of hemorrhage and necrosis, with increased lipoid content of the cells and lymphocytic infiltration, were noted in the adrenal glands. In the brain there were many petechial hemorrhages adjacent to thrombotic occlusions of small arterioles and capillaries (fig. 2). No polymorphonuclear exudate, inclusion bodies or meningeal inflammatory reaction or other specific incriminating lesions were observed. Early astrocytic and oligodendroglial swelling were the only prominent glial changes.

As the distinctive pathologic feature was the hyaline thrombotic material within small vessels, particularly those of the brain, a composite description will be given.

Description of the Thrombotic Lesion: Precapillary arterioles, capillaries and venules all showed this unique lesion. A few of the arterioles showed smudgy walls retaining a bright eosinophilic stain. This was noted particularly in vessels of the brain. No mural or extramural inflammatory exudate was encountered in the

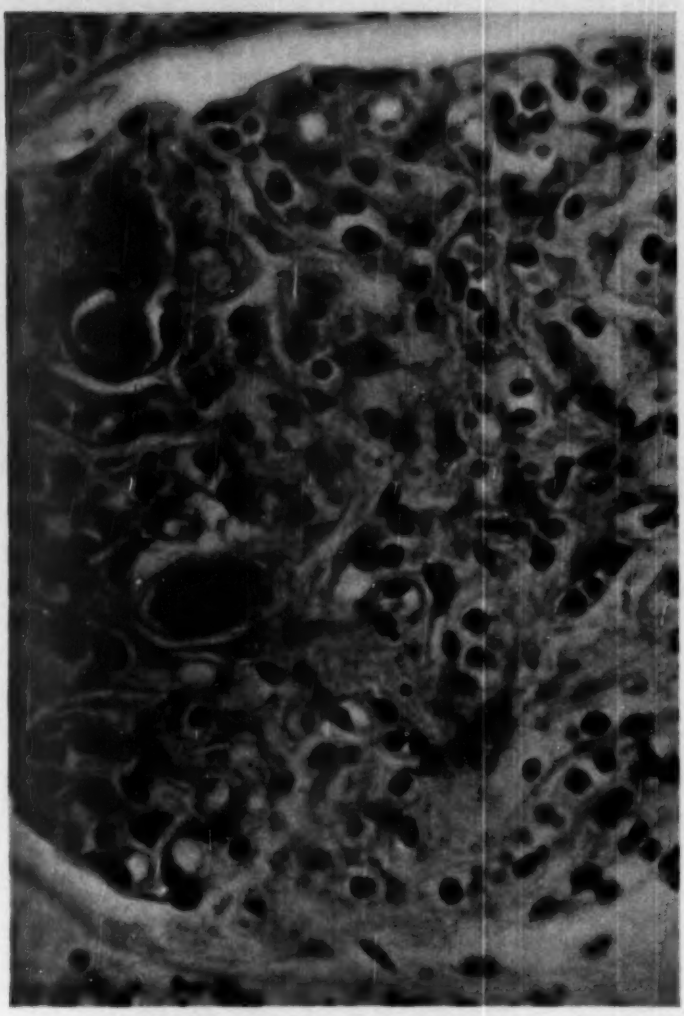

Fig. 1.—Renal glomerulus showing endothelial proliferation and two intracapillary hyaline thrombocytic thrombi (× 620).

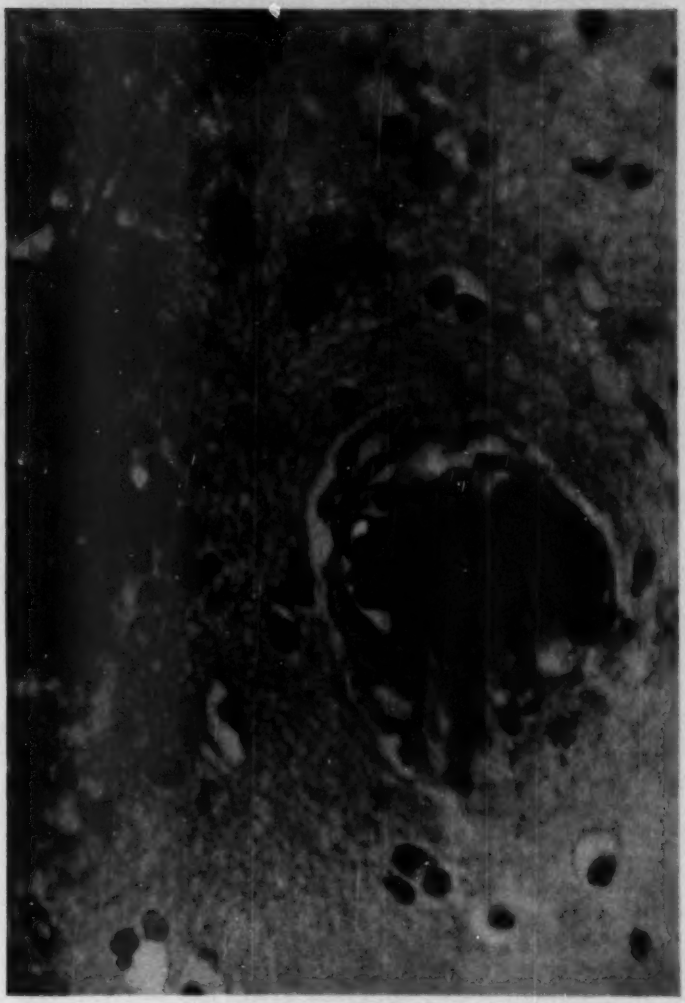

Fig. 2.—Brain with a blood vessel occluded by an amorphous hyaline thrombus and a fresh hemorrhage in the vicinity (×480).

thrombosed vascular channels. Inclusion bodies such as occur in tropical phlebitis [7] were not observed. The thrombotic matrix stained as follows: hematoxylin-eosin, pink; amyloid stain, negative; prussian blue, negative; Weigert's fibrin stain, negative; methyl green-pyronin, purple; thionine, blue-red; hemoglobin stain, negative. These tinctorial reactions were interpreted as excluding a red cell or fibrin derivation and as being more in keeping with a thrombocytic origin. The thrombocytic material was nonlamellated and granular, and this granularity was more pronounced in the center of the matrix. In some of the lesions the thrombotic material extended under the endothelium. Within the vessels of the brain and the kidney there was considerable overlying endothelial proliferation. The endothelial nuclei were pleomorphic but retained an alinement or enveloping position around the thrombi. The thrombotic masses were enfiladed by endothelial cells, with little or no organization from the base of the lesion.

The outstanding alteration was the widespread formation of intravascular thrombi in the small vessels, responsible for the pathologic findings of hemorrhagic encephalopathy and possibly the severe nephrosis. Originally it was considered that these disseminated thrombocytic thrombi indicated lesions produced by heavy metal poisoning. This was ruled out by the clinical history and the chemical analyses of the kidneys, which were negative for arsenic, lead, copper, mercury and tin. The final anatomic diagnosis was hemorrhagic encephalopathy; thrombopenic purpura; hyaline thrombosis of the arterioles, capillaries and venules of the brain, heart, kidney, bone marrow and pancreas; acute proliferative glomerulonephritis; severe nephrosis, and focal necrosis of the liver and the adrenal glands.

COMMENT

Since 1925 there have appeared in the American literature reports of 21 cases of thrombotic thrombopenic purpura. In a number of these cases clinical neurologic signs and symptoms were indicative of an encephalitis of undetermined origin. All the patients were acutely ill with fever, unusually pale and had an underlying icteric tint. Ecchymoses and petechiae were noted over the body surfaces. Neurologic changes were frequent in the form of either mental confusion, stupor, coma and convulsions or, on occasion, baffling lateralizing signs. Laboratory study frequently revealed red blood cells in the urine, with increased urobilinogen, but no bile. Profound anemia and pronounced thrombopenia have been the cardinal features of the hematologic studies. The majority of the reported cases have shown all these features at some time during the course of the fatal illness. There is no doubt that the complexity of the clinical picture has rendered antemortem diagnosis difficult, but an encephalopathy of sudden onset and baffling nature with persistent fever, low thrombocyte count and severe anemia should suggest this unique disorder.

A question immediately arises as to the nature of the intravascular thrombotic material. For years, hyaline thrombi have been interpreted as of red cell origin. Red cell thrombi have been produced experi-

7. Fisher, A. C.; Fisher, M. M., and Lendrum, A. C.: J. Path. & Bact. 59:405, 1947.

mentally in the past by injection of foreign blood, potent hemolysins or heavy metals. Baehr, Klemperer and Schifrin [8] departed from this conception in 1936 and presented 4 cases akin to this one with the assertion that these hyaline amorphous thrombi were derived from thrombocytes. All recent writers on this entity except Moschowitz [9] have attributed the thrombotic material to thrombocytic breakdown. Moschowitz, who originally described this disorder, expressed the belief that the material was derived from red cell degradation products. Consideration of a red cell genesis of the vasothrombosis is eliminated because of the negative hemoglobin stains and the absence of a potassium ferrocyanide reaction, and, finally, the chemical analyses carried out on the organs failed to reveal any evidence of heavy metal poisoning.

In 1948 Gore [10] described 5 cases of this disease and asserted that the basic fault lay within the intercellular cement substance of the small vessels. Through interpretation of a periodic acid staining reaction, he came to the opinion that focal swelling is the initial alteration of the vessel wall; endothelial proliferation and thrombocytic deposition are morphologic sequels. The thrombocytic thrombotic material may be regarded as the morphologic reflection of a "sealing off" of the regions of disrupted or damaged vessels. Closer study reveals that the peripheral portions of the thrombi are composed of amorphous hyaline material, of mural origin. The core of luminal material is granular and made up of a fused aggregation of thrombocytes. Because the endothelial cells have proliferated, forming sleeves over the thrombotic masses, with no organization of these thrombi from their point of attachment, it is suggested that the endothelial lining has been disrupted by the primary vascular defect. Although Zucker,[11] in his studies on blood clotting, concluded that thrombocytic thrombosis does not occur in idiopathic thrombopenic purpura and denied, furthermore, the existence of thrombocytic thrombi in capillaries, it is still the most likely explanation that the constituents of the hyaline thrombi are fused masses of thrombocytes.

The genesis of the original defect, whether in the vascular wall or in the sudden precipitation and agglutination of thrombocytes, is not known. A satisfactory explanation of the sudden hemolytic anemia which accompanies the thrombopenia has not as yet been advanced. Certainly the hemosiderosis and the blood extravasated into tissues through capillary defects will not explain the profound fall of hemoglobin.

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8. Baehr, G.; Klemperer, P., and Schifrin, A.: Tr. A. Am. Physicians **51**:43, 1946.

9. Moschowitz, E.: Proc. New York Path. Soc. **24**:21, 1924.

10. Gore, I., and Friedman, N. D.: Proc. Am. A. Path. & Bact., March 1948, p. 703.

11. Zucker, H. D.: Blood **4**:631, 1949.

Although the bone marrow contains intravascular thrombi identical in appearance with those found in other viscera, it is apparent that this is an incomplete vascular occlusion, and because of the segmental nature of the lesion, a significant necrosis of the marrow elements is unlikely to result. Certainly, alterations of the thrombocytic progenitors are not seen; hence, the purpura cannot be explained on the basis of a deficient production of thrombocytes.

The thrombopenia may be accounted for on the basis that many thrombocytes are combed out of the circulation and enmeshed in the thrombosed vessels, with quantitative exhaustion of the thrombocyte supply. The bleeding tendencies necessitate the assumption of capillary endothelial damage. Achard and Aynaud [12] and Hanzlick and Karsner [13] have shown that in experimental shock there is a rapid disappearance of the thrombocytes. This peripheral depletion of thrombocytes is accounted for by their presence as agglutinates in the small capillaries of the internal organs. Eberth and Schimmelbusch [14] pointed out that when the circulation slackens the thrombocytes collect at the periphery of the stream and by passive means seal off the defects of the vascular bed.

Some unrecognized form of vascular idiosyncrasy may be the fundamental defect. Capillary proliferation is the alteration on which much of the evidence for hypersensitivity rests. Trobaugh, Markowitz, Davidson and Crowley [15] Carter,[16] Altschule [17] and other authors [18] favor a hypersensitivity provenance, principally because of the occurrence of endothelial proliferation without thrombosis. Muirhead, Grass and Hill [6c] interpreted the proliferative glomerulonephritis as morphologic support for the tenet that the endothelial proliferation was the lesion of primacy. Carter [16] and Gore [10] stressed the mural vascular damage. Neither the endothelial proliferative lesions nor the mural defects are accompanied by a neutrophilic inflammatory response, which is evidence against bacterial infection being responsible for this disorder. With the vascular involvement being segmental and the thrombotic intravascular desposits in various phases of healing, the morphologic interpretation is indicative of repeated episodes of thrombocyte-vessel assault, probably antigenic.

12. Achard, C., and Aynaud, M.: Compt. rend. Soc. de biol. **65**:554, 1908.
13. Hanzlick, P., and Karsner, H. T.: J. Pharmacol. & Exper. Therap. **23**:237, 1924.
14. Eberth, C. J., and Schimmelbusch, C.: Virchows Arch. f. path. Anat. **101**:201, 1885.
15. Trobaugh, F. E.; Markowitz, M.; Davidson, C. S., and Crowley, W. F.: Arch. Path. **41**:327, 1946.
16. Carter, J. R.: Am. J. M. Sc. **213**:585, 1947.
17. Altschule, M. D.: New England J. Med. **227**:477, 1942.
18. Gitlow, S., and Goldmark, C.: Ann. Int. Med. **13**:1046, 1939.

Ehrich and Seifter [19] recently reported a case of thrombotic thrombopenic purpura; they attributed the thrombopenia to an antigen, iodine, which had been administered before death and which evoked antibodies. According to his concept, this combination of antigen-antibody led to thrombocytic agglutination and then anoxia of the distal vascular endothelium, with the production of clinical purpura. Experimental work supporting the proponents for primary vascular damage has been extrapolated from the research of Schwartzman.[20] Goddard [21] has induced endothelial hyperplasia and proliferation in sensitized animals with specific antigens; interestingly enough, venules, precapillary arterioles and capillaries all shared in this response. If this is true, an antigen should be sought for in all future cases of this disorder.

Although certain features suggest a linkage of this and the hypersensitive collagen diseases, as yet the existence of purpuric blotches and generalized vasothrombotic changes is insufficient evidence on which to postulate an intimate clinical association with disseminated lupus erythematosus. Klemperer [22] recently stated that this peculiar condition is not related to disseminated lupus. Although its origin and nature are still obscure, it is believed to be a definite entity. Owing to the widespread formation of thrombocytic thrombi, the central nervous system is frequently affected, and because of the morphologic intimacy between vessels and thrombocytes, an antigen-antibody response may be at fault.

SUMMARY

A case of hemorrhagic encephalopathy of disseminated vasothrombotic origin intimately associated with a thrombocyte-poor purpura is reported. The etiologic, anatomic, pathologic and clinical features are discussed, with emphasis on the antigenic nature of the disorder.

19. Ehrich, W. E., and Seifter, J.: Arch. Path. **47**:446, 1949.

20. Schwartzman, G.; Klemperer, P., and Gerber, I. E.: J. A. M. A. **107**: 1946, 1936.

21. Goddard, J. W.: Am. J. Path. **23**:943, 1947.

22. Klemperer, P.: Proc. Am. A. Path. & Bact., March 1948, p. 704.

HISTOCHEMICAL STUDIES OF SKIN WOUNDS IN NORMAL AND IN SCORBUTIC GUINEA PIGS

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THE LESION of scurvy has been demonstrated to be an interference in the formation of collagen, which serves as the common substructure for such intercellular supporting tissues as bone, cartilage, fibrous connective tissue and dentin.[1] Much investigation has been directed toward the study of wound healing in the ascorbic acid–deficient state. The wounds are observed to heal poorly and slowly with a closure that is easily disrupted because the collagen formation is inadequate even though extensive proliferation of fibroblasts exists.[2] Danielli, Fell and Kodicek [3] found that the capillaries, fibroblasts and intercellular material of the wounds of scorbutic animals were deficient in alkaline phosphatase in contrast to those of normal animals and concluded that the enzyme was associated in some unknown manner with collagen formation. Their findings have been confirmed in the present investigation. In addition, observations have been made on the acid mucopolysaccharides of healing wounds of scorbutic and normal guinea pigs as related to the state of fibroblastic proliferation and collagen formation.

METHODS

This study was conducted on 20 guinea pigs weighing approximately 250 Gm. each. An ascorbic acid–free diet [4] was given *ad libitum* to 10 experimental animals.

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1. (*a*) Höjer, J. A.: Acta pædiat. (supp. 3) **3**:1 1924. (*b*) Wolbach, S. B., and Howe, P. R.: Arch. Path. **1**:1, 1926.

2. Wolbach, S. B.: Am. J. Path. **9**:689, 1933. Lanman, T. H., and Ingalls, T. H.: Ann. Surg. **105**:615, 1937.

3. Danielli, J. F.; Fell, H. B., and Kodicek, E.: Proc. Nutrition Soc., 1946, p. 197.

4. Cod liver oil, 1 Gm.; dextrose, 36 Gm.; casein, 30 Gm.; celluflour,* 20 Gm.; liver fraction L, 7 Gm.; salts IV, 5 Gm.; fortified corn oil, 1 Gm.; choline chloride

The 10 control guinea pigs received the same diet supplemented with 15 mg. of ascorbic acid to each 100 Gm. of ration and, in addition, each was given 15 mg. of ascorbic acid per day plus greens *ad libitum*.

The experimental wounds were produced after both groups of animals had been fed their respective diets for two weeks. At this time signs of scurvy were indicated during life by the animals' listlessness, failure to gain or actual loss in weight, tenderness and swelling about the joints, bleeding gums and loosened incisors, and at autopsy by small hemorrhages at the costochondral junctions and conspicuous reduction of adrenal cortical lipid as compared with the controls.

The wounds consisted of clean scalpel incisions made through the skin of the back, which had previously been shaved and sterilized. Animals were killed at intervals up to twenty-six days thereafter; the tissues were fixed and stained in the following ways. Zenker's fluid was employed as the fixative when tissues were stained with eosin–methylene blue or methylene blue alone for basophilia; it was also used when they were prepared with Mallory's trichrome connective tissue stain and Laidlaw's silver method for reticulin and collagen. Alkaline phosphatase was demonstrated by Gomori's [5] method, cold 80 per cent alcohol being used as fixative and glycerophosphate at a p_H of 9.1 as substrate; incubation was carried out at 37 C. for twenty-four to forty-eight hours; shorter periods were found insufficient for optimal results, confirming the observation of Fell and Danielli.[6] The relative amount of the resulting cobalt sulfide in each preparation was estimated and recorded.

The acid mucopolysaccharides of the tissues were fixed with 4 per cent basic lead acetate. They were detected by subsequent metachromatic staining with toluidine blue in conjunction with the use of hyaluronidases as described toward the end of this paragraph.[7] The toluidine blue was used in a 0.05 per cent aqueous solution (p_H 4.5 to 5.0) at room temperature for about eighteen hours; the slides were dehydrated by gentle agitation for three seconds each in 95 per cent and absolute alcohol and then placed directly in xylene and mounted. In this way uniformity of preparation was obtained for purposes of comparison. The amount in each was estimated and recorded by means of plus signs as indicated in the table. Hyaluronidases of testicular and streptococcic origin (supplied by Wyeth Research Laboratory of Wyeth, Incorporated, Philadelphia) were employed at a concentration of 15 turbidity reducing units per cubic centimeter in 0.3 per cent sodium chloride

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and inositol, 100 mg. to each 100 Gm. of ration; thiamine hydrochloride, and pyridoxine hydrochloride, 0.2 mg. to each 100 Gm. of ration; riboflavin, 0.5 mg. to each 100 Gm. of ration; nicotinic acid and calcium pantothenate, 1.0 mg. to each 100 Gm. of ration; corn oil fortified to provide 2,000 units of vitamin A, 100 units of vitamin D and 0.004 Gm. of alpha tocopherol, to each 100 Gm. of ration. This diet was supplied through the courtesy of Dr. D. Mark Hegsted, of the Department of Nutrition, Harvard University. "Salts IV" is described elsewhere (Hegsted, D. M.; Mills, R. C.; Elvehjem, C. A., and Hart, E. B.: J. Biol. Chem. **138**:459, 1940). Cellufiour® is a specially treated cellulose, completely indigestible, used to supply roughage, marketed by the Chicago Dietetic Supply House, Chicago. Liver fraction L is supplied by Wilson Laboratories, Division of Wilson & Co., Inc., Chicago.

5. Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941.

6. Fell, H. B., and Danielli, J. F.: Brit. J. Exper. Path. **24**:196, 1943.

7. Wislocki, G. B.; Bunting, H., and Dempsey, E. W.: Am. J. Anat. **81**:1, 1947.

(p_H 6.0 to 6.5) at 37 C. for eighteen hours; deparaffinated sections subjected to the action of either enzyme were then washed and stained with toluidine blue along with a duplicate exposed under similar conditions to boiled enzyme solution.

Glycogen in the tissues was preserved by cold Rossman's fluid (saturated alcoholic picric acid solution to which 40 per cent formaldehyde was added to make 5 per cent of the total); glycogen was detected by the Bauer method[8] comparing a section that had been digested for two hours in a 0.2 per cent diastase solution in sixtieth-molar potassium dihydrogen phosphate (p_H 5) with one that had been similarly incubated in buffer alone.

Cytoplasmic ribonucleotides were detected by the action of ribonuclease. Crystalline ribonuclease (Armour & Company) was treated as recommended by McDonald[9] to destroy the proteolytic activity. Sections were exposed to it in a solution of 0.2 mg. per cubic centimeter at 60 C. for four hours. Controls were similarly exposed to buffer alone. Both sections were then stained with a 0.003 per cent solution of methylene blue for two and a half hours, dipped quickly in water, air dried, dipped momentarily in absolute alcohol and placed in xylene until cleared, and then mounted. By comparing the control slide with that incubated in the enzyme, regions of basophilia removed by the latter could be detected. The relative incidence of mitoses in the regenerating fibroblasts was evaluated by counting those found in six high power fields chosen at random in samples of each wound. The fibroblasts were judged "immature" or "adult" by cytologic features: Cells were considered "immature" if their nuclei were large and vesicular and their cytoplasm abundant; or "adult" if the nuclei were smaller and the cytoplasm scanty. The relative number of each type that fell in either of these categories was then estimated and recorded. Intercellular connective tissue fibers staining black with silver were arbitrarily designated reticulin (or precollagen), while those staining brown were called collagen. Mallory's connective tissue stain was also used for confirmation of the latter. Collagen was rated in a roughly quantitative manner in three categories: fine, medium and coarse. It should be noted that while the quantitative evaluations recorded undoubtedly contain some subjective element, the identity of the slide was unknown at the time the estimate was being made.

RESULTS

Many of the findings are presented in detail in the table.

Differences were evident between the wounds of the scorbutic and the normal animals. While some intercellular connective tissue was formed in both groups, its character was not the same in each. In the controls the collagen as first formed was in fine strands; these were later transformed into much coarser strands coincidentally with the changing of the large fibroblasts to smaller adult or resting cells (fig. 1 *A*, *B* and *C*). In the scorbutic animals, while strands stainable as collagen were formed, they persisted as fine or at most as medium-sized fibers for the duration of the period of observation; the connective tissue cells were predominantly large and immature in type, and mitoses were

8. Lison, L.: Histochimie animale: Méthodes et problèmes, Paris, Gauthier-Villars, 1936.

9. McDonald, M. R.: J. Gen. Physiol. **32**:39, 1948.

Detailed Observations in Individual Animals

Age of Wound, Days	Fibroblasts of Wound: Mitoses: Control	Fibroblasts of Wound: Mitoses: Scorbutic	Fibroblasts of Wound: Type of Cells: Control: Imm.	Fibroblasts of Wound: Type of Cells: Control: Adult	Fibroblasts of Wound: Type of Cells: Scorbutic: Imm.	Fibroblasts of Wound: Type of Cells: Scorbutic: Adult	Connective Tissue Fibers: Control: Reticulin	Connective Tissue Fibers: Control: Collagen: Fine	Connective Tissue Fibers: Control: Collagen: Med.	Connective Tissue Fibers: Control: Collagen: Coarse	Connective Tissue Fibers: Scorbutic: Reticulin	Connective Tissue Fibers: Scorbutic: Collagen: Fine	Connective Tissue Fibers: Scorbutic: Collagen: Med.	Connective Tissue Fibers: Scorbutic: Collagen: Coarse	Metachromasia of Wound: Control	Metachromasia of Wound: Scorbutic	Alkaline Phosphatase of Wound: Control	Alkaline Phosphatase of Wound: Scorbutic
3	4	..	+++	0	.....	..	.....	.....	....	....	.....	....	....	..	++	+++	....	....
7	1	13	++	++	+++	0	+++	++	0	0	+++	+	0	0	+	+++	+	0
11	0	2	+	+++	+++	0	++	+++	++	0	+++	++	0	0	+	+++	+	++
14	..	3	..	.....	+++	+	.....	.....	.....	.....	+++	++	0	0	.....	.....	.....	0
16	1	0	0	+++	+++	+	+	+++	+++	0	+++	+	0	0	tr	++	++	0
18	0	1	0	+++	+++	+	.....	.....	.....	.....	+	+++	+++	0	+	+	+++	+++
20	0	1	0	+++	+++	+	++	+++	+++	0	+++	0	+	0	++	+	+++	++
22	1	2	+	+++	+++	0	+	0	+++	+++	+++	+++	++	0	+	+++	+++	0
24	0	0	0	+++	+++	+	++	0	++	+++	+++	++	++	0	+	+++	+++	0
28	0	1	0	+++	+++	0	tr	0	+++	+++	+++	0	+	0	++	+++	+++	0

Key: 0, tr (trace) and plus sign indicate estimated degree or amounts present; number of mitoses counted in 6 high power fields as recorded; Imm. indicates immature type of fibroblast.

uniformly more numerous than in the control wounds (fig. 1 *D*). Cytoplasmic basophilia, conspicuous in the fibroblasts of both groups, was presumably due to ribonucleotides since it could be removed by ribonuclease. After being treated with toluidine blue, the cytoplasm of the fibroblasts was at times slightly metachromatic; this effect was also removed by ribonuclease, suggesting that this staining reaction, also,

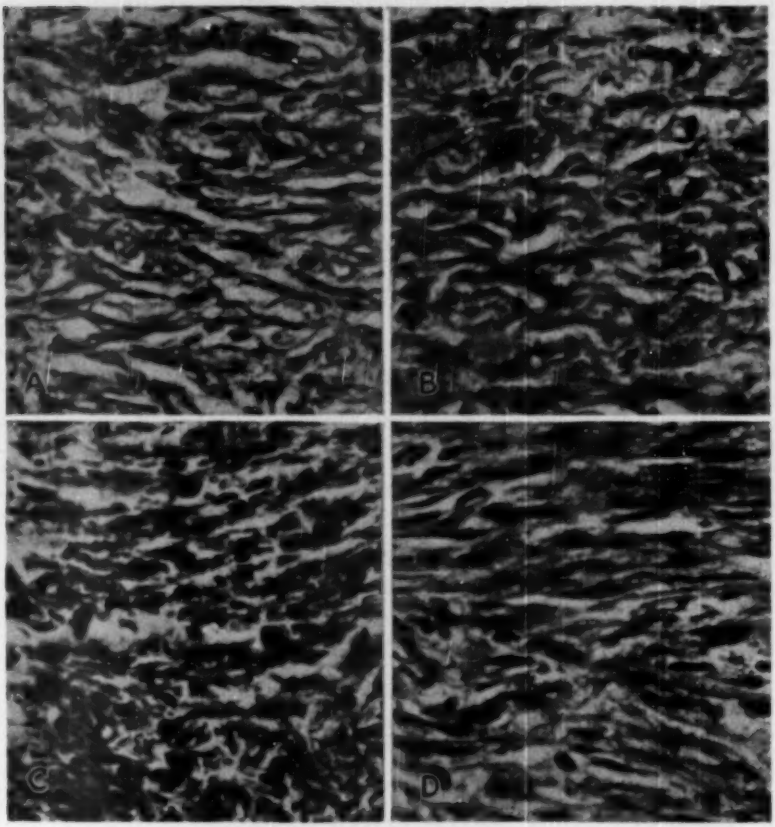

Fig. 1.—*A*, control, eleven day wound; Mallory connective tissue stain. Some immature but chiefly adult-appearing fibroblasts; fine and medium thick collagen fibers. × 660.

B, control, sixteen day wound; same stain and magnification as in *A*. Greater proportion of adult-appearing fibroblasts than in *A*.

C, control, twenty-two day wound; same stain and magnification as in *A* and *B*. Fibroblasts predominantly adult-appearing; medium thick and coarse strands of collagen.

D, scorbutic animal, twenty-two day wound; same stain and magnification as in *A*, *B* and *C*. Predominance of immature-appearing fibroblasts and fine and medium thick strands of collagen.

resulted from the presence of ribonucleotides.[7] While argyrophilic fibers were present in the wounds of both groups, they were much more numerous throughout the entire scorbutic series (fig. 2 *A* and *B*). These fibers by definition would be designated reticulin or in this particular instance precollagenous reticulin.

Material that stained metachromatically with toluidine blue was present in the wounds of both groups of animals; it was especially conspicuous in the early examples. The metachromatic material lay between the fibroblasts and the capillaries, appearing to coat the connective tissue fibers. When the section was exposed to hyaluronidase of testicular origin prior to staining with toluidine blue, no metachromasia resulted,

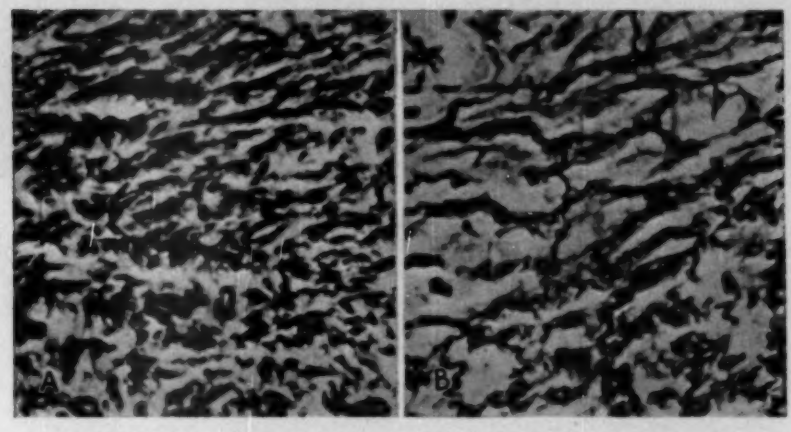

Fig. 2.—*A*, control, twenty-six day wound; Laidlaw's silver stain; same magnification as in figure 1. Abundance of connective tissue strands, almost all collagen, very little reticulin.

B, scorbutic animal, twenty-six day wound; same stain and magnification as in *A*. Abundant reticulin fibers and some medium thick collagen.

leading to the conclusion that the substance responsible for this staining was probably a mucopolysaccharide. Hyaluronidase of streptococcic origin, however, did not have this effect. The metachromatic material in these wounds resembled that of sites known to be rich in chondroitin sulfate (e.g., hyaline cartilage).[10] The metachromasia of the granules of the mast cells in the dermis was unaffected by either enzyme, but the metachromasia present in small amounts in the epidermal basement membrane and papillary layer of the adjacent uninjured skin behaved similarly to that in the wounds. While metachromatic material was

10. Bunting, H.: Proc. New York Acad. Sc., to be published.

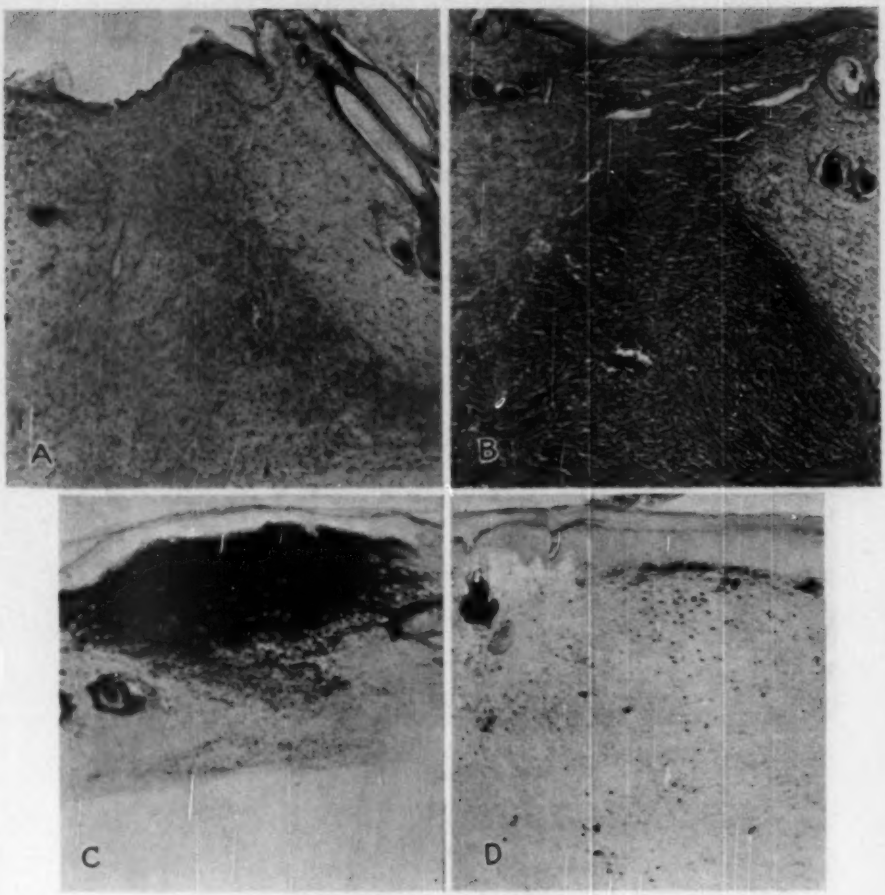

Fig. 3.—*A*, control, twenty-six day wound; slight metachromasia of connective tissue of wound following staining with toluidine blue. (Compare with *B*.) × 75.

B, scorbutic animal, twenty-six day wound; same stain and magnification as in *A*. Marked metachromasia of connective tissue of wound.

C, control, twenty-four day wound; alkaline phosphatase abundant in fibroblasts and collagen of wound. × 75.

D, scorbutic animal, twenty-four day wound; absence of alkaline phosphatase in fibroblasts and collagen of wound; hair follicle at left shows alkaline phosphatase. × 75.

present in wounds of both groups at all stages studied, it was apparent that the wounds of the scorbutic animals in all but 1 instance had larger amounts than those of comparable controls (fig. 3 *A* and *B*).

Mast cell counts were made on the regenerating connective tissue of the wounds: The cells were frequently variable in number, ranging from none to 6 per high power field in any one wound. Mast cells could be found within, as well as entirely separate from, the areas of metachromasia. No correlation was found between the average mast cell count and the degree of metachromasia of the connective tissue of the wounds as a whole.

Alkaline phosphatase was first seen in association with the polymorphonuclear leukocytes accumulated at the surface of the wound. Later, in the control wounds the enzyme was demonstrable in the capillary endothelium, in the nuclei and the cytoplasm of the fibroblasts and on the actual collagenous strands, where it persisted without much apparent quantitative change for the remainder of the observation period. In the scorbutic wounds, the occurrence of the enzyme was irregular; it was present in moderate concentration in 3 instances, but was not demonstrable in the remainder (fig. 3 *C* and *D*).

Glycogen was found in the polymorphonuclear leukocytes of the crusts of fibrin and exudate that covered the wound soon after it was made, and very irregularly in the proliferating fibroblasts of the wound, in both scorbutic and control animals. The regenerated epidermis that covered the actual site of the wound in both groups was thicker than elsewhere, containing 4 or 5 cells instead of 2 or 3. Glycogen was present in the cells of the malpighian layer where it was thicker, but not in the surrounding epidermis. The cells of the regenerated epidermis were more basophilic than those of the resting epidermis. The basophilia was most evident in the lower layers of cells, but was also recognizable to a lesser extent in the upper portion of the epidermis. Ribonuclease removed the substance responsible for this basophilia, allowing one to conclude that the basophilia was probably due to ribonucleotides.

COMMENT

Wolbach and Howe [15b] held that no intercellular material whatever is formed in a state of absolute scorbutus. If one accepts this criterion, the present scorbutic group was in a state of relative scorbutus even though the animals had received no dietary ascorbic acid for fourteen days prior to the incision and none at any time thereafter for the duration of the experiment. The observations will therefore be interpreted on this basis.

The wounds of the relatively scorbutic guinea pigs differed from those of the control guinea pigs in several respects. In the wounds of the controls precollagenous reticulin and fine collagen strands were formed early; the reticulin soon diminished, while the collagenous

strands became heavier, producing a dense scar. The fibroblasts were actively proliferating in the early lesions but were slowly transformed into fairly mature cells with small nuclei and less abundant cytoplasm. The wounds of the partially scorbutic animals differed from those of the controls in having a larger amount of precollagenous reticulin, which persisted throughout the observation period of twenty-six days. The collagen, which was laid down in small or moderate amounts, was present in fine fibers which later became somewhat heavier but which in no instance were comparable with the dense collagen of the mature scars of the controls; even in wounds more than three weeks old the fibroblasts persisted as large cells with abundant cytoplasm. In brief, the late wounds of the partially scorbutic guinea pigs were composed of immature-appearing fibroblasts and reticular and collagenous strands resembling those found in the early wounds of normal animals.

Danielli, Fell and Kodicek [9] observed that alkaline phosphatase was absent from wounds of scorbutic guinea pigs and present in those of normal ones, and that it was capable of being partially restored by administration of small amounts of ascorbic acid; it was suggested that the enzyme was related in some way to collagen formation. Our observations in general confirm theirs. Individual animals of the partly scorbutic group showed wide differences in the amount of enzyme present, although all exhibited about the same interference in collagen production. This suggested that there was no causal relationship between these, but that both constituents were altered independently in scurvy. The alkaline phosphatase of the kidney and the serum are also known to be reduced in this condition.[11]

There was abundant metachromatic substance in the connective tissue of the wounds of both groups of guinea pigs. While there was some individual variation, the wounds of the partly scorbutic animals had an abundance of metachromasia exceeding that of comparable controls in all but 1 instance. The metachromatic substance appeared to be concentrated on the connective tissue fibers, but this may well be an artefact induced by fixation, as was the case in the umbilical cord where the metachromatic material was present as a diffuse ground substance in the fresh state.[10]

Sylvén [12] concluded that the metachromatic substance of healing wounds is derived from mast cells by discharge of the content of their granules. This he based on the observation that the mast cells were fewer or had fewer granules where the metachromasia was greatest, suggesting either a total or a partial loss of their contained granular

11. Russell, W. O.; Rouse, L. T., and Read, J. A.: Arch. Path. **38**:40, 1944. Shwachman, H., and Gould, B. S.: J. Nutrition **23**:271, 1942.

12. Sylvén, B.: Acta chir. Scandinav. (supp. 66) **86**:1, 1941.

material. In the present material the abundance or the scarcity of mast cells could not be correlated with metachromasia of the tissue. Further, the use of testicular hyaluronidase indicated a difference in the chemical composition of the metachromasia at the two sites, since it eliminated that of the connective tissue without affecting that of the mast cell granules. An alternative proposal is that fibroblasts (and mesenchymal cells) are capable of elaborating the mucopolysaccharides. The evidence for this at present is only circumstantial; e. g., an active functional state of these cells is correlated with the presence of abundant mucopolysaccharides as in newly formed connective tissue in general, or during the proliferative phase only of the endometrium [13]; the mucopolysaccharides occur in a halo immediately surrounding the isolated fibroblasts within the relatively acellular dense connective tissue of the sclerotic aortic wall.[14] The response of the metachromasia to the hyaluronidases in all these sites has been the same.

The metachromatic substance found in the wounds of both groups of animals (as well as in the normal dermis) behaves similarly to that in sites high in chondroitin sulfate,[15] since it is susceptible to the testicular but not to the streptococcic hyaluronidase. It is known that testicular enzyme affects mucopolysaccharides containing sulfate as well as those without, while streptococcic affects the latter group only.[14]

These experiments shed little light on the function performed by the acid mucopolysaccharides in the developing connective tissue. Meyer [15] has postulated that they might be related to the actual formation of collagen. This would be consistent with the observations made on this subject by Penney and Balfour [16] in an excellent study that has appeared since the completion of the present report. These investigators found no mucopolysaccharides in the mass of proliferating fibroblasts of wounds when the animals were completely scorbutic. Following administration of small amounts of ascorbic acid, abundant mucopolysaccharides appeared in the wounds simultaneously with some intercellular fibers.

The abundance of cytoplasmic ribonucleotides in the regenerating fibroblasts and epidermal cells is to be correlated with the relatively high

13. Bartelmez, G. W., and Bensley, C. M., in Cowdry, E. V.: Special Cytology: The Form and Functions of the Cell in Health and Disease, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 1523. Bensley, S. H.: Anat. Rec. **60**:93, 1934. Wislocki, G. B.; Bunting, H., and Dempsey, E. W., in Engle, E. T.: Menstruation and Its Disorders, Springfield, Ill., Charles C Thomas, Publisher, 1950, p. 23.

14. Meyer, K.; Chaffee, E.; Hobby, G. L., and Dawson, M. H.: J. Exper. Med. **73**:309, 1941.

15. Meyer, K.: Am. J. Med. **1**:676, 1946.

16. Penney, J. R., and Balfour, B. M.: J. Path. & Bact. **61**:171, 1949.

protein synthetic activity of these growing cells.[17] In both instances there is rapid formation of cytoplasm of the regenerating cells while the fibroblasts, in addition, are engaged in the elaboration of the fibrous protein collagen. Keratin is, of course, also being formed by the epidermal cells, but there is no evidence that this activity is increased beyond the normal in this zone.

The metabolism of the regenerating cells of the epidermis would appear to be different from that of the "resting" ones inasmuch as cytoplasmic glycogen is demonstrable in the former only. What relationship there may be between this finding and the metabolic needs cannot be conjectured from the information at hand.

SUMMARY

The occurrence of alkaline phosphatase and acid mucopolysaccharides has been studied in skin wounds of partially scorbutic and normal guinea pigs. The findings have been related to the state of fibroblastic proliferation and the formation of intercellular material. In general, healing was retarded in partial scurvy: the wounds contained immature-appearing fibroblasts and intercellular material which resembled that of earlier wounds of normal animals, being rich in reticulin and relatively deficient in collagen. The alkaline phosphatase of fibroblasts and intercellular material was absent or greatly reduced in the wounds of the scorbutic group, but there was no correlation between the amounts of this enzyme demonstrable and the collagen present in individual animals. Acid mucopolysaccharides were more abundant in the connective tissue of the wounds of partially scorbutic than in normal animals; and these mucopolysaccharides reacted to streptococcic and testicular hyaluronidases as to those in sites known to be rich in chondroitin sulfate.

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17. Caspersson, T.: For review of his and his associates' studies, cf.: Symp. Soc. Exper. Biol., 1947, no. 1, p. 127. Brachet, J.: Arch. de biol., Paris **53**:207, 1942.

Case Reports

ADDISON'S DISEASE FROM AMYLOIDOSIS OF ADRENAL GLANDS

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AND
SAMUEL J. CAMARATA, M.D.
PITTSBURGH

AMYLOID involvement of the adrenal glands of sufficient extent to produce the clinical syndrome of Addison's disease from cortical insufficiency is a rare manifestation of amyloidosis. In the vast majority of cases Addison's disease results from either active bilateral tuberculous destruction of the glands or from idiopathic atrophy. In a review of 566 reported cases of Addison's disease, Guttman [1] found amyloid disease as the etiologic factor in only 7 cases—an incidence slightly greater than 1 per cent.

In view of the frequency with which the adrenal cortex is involved in generalized amyloidosis, it is surprising that cortical insufficiency is not encountered more frequently. Probably, bilateral amyloid deposition extensive to the point of almost total replacement of cortical tissue is requisite before insufficiency becomes clinically manifest as in the following case.

REPORT OF CASE

D. T., a 40 year old white woman, was admitted to the service of Dr. George E. Martin of the City Tuberculosis Hospital on March 20, 1947, with the complaints of fever, a productive cough and weakness. She had been hospitalized three times for chronic pulmonary tuberculosis since 1925. Physical and radiologic examination revealed bilateral pulmonary tuberculosis with cavitation. Laboratory studies disclosed moderate anemia, increased sedimentation of erythrocytes to 29 mm. per hour and sputum positive on direct smear for tubercle bacilli. The temperature on admission was 102 F.

Under a standard regimen there was progression of her pulmonary infection during the first six months of hospitalization. In the following six months a brownish pigmentation gradually developed about the forehead and neck and became progressively more intense, involving the entire face, the thorax and the thighs. This pigmentation had characteristics typical of Addison's disease, and at death the buccal margin and oral mucous membrane showed a distinct bluish discoloration. The weakness became more severe and eventually progressed to the point, a few weeks before death, where the patient was scarcely able to raise her arms. Her voice became extremely weak and high pitched, and even talking became an effort. There was a loss of weight from 104 pounds (47 kilograms) at the time of admission to 68 pounds (about 31 kilograms) at death. Two months before death, abdominal cramps and severe diarrhea developed and became progressively worse. The

From the Department of Pathology, University of Pittsburgh, and City Tuberculosis Hospital.

1. Guttman, P. H.: Arch. Path. **10**:742, 1930.

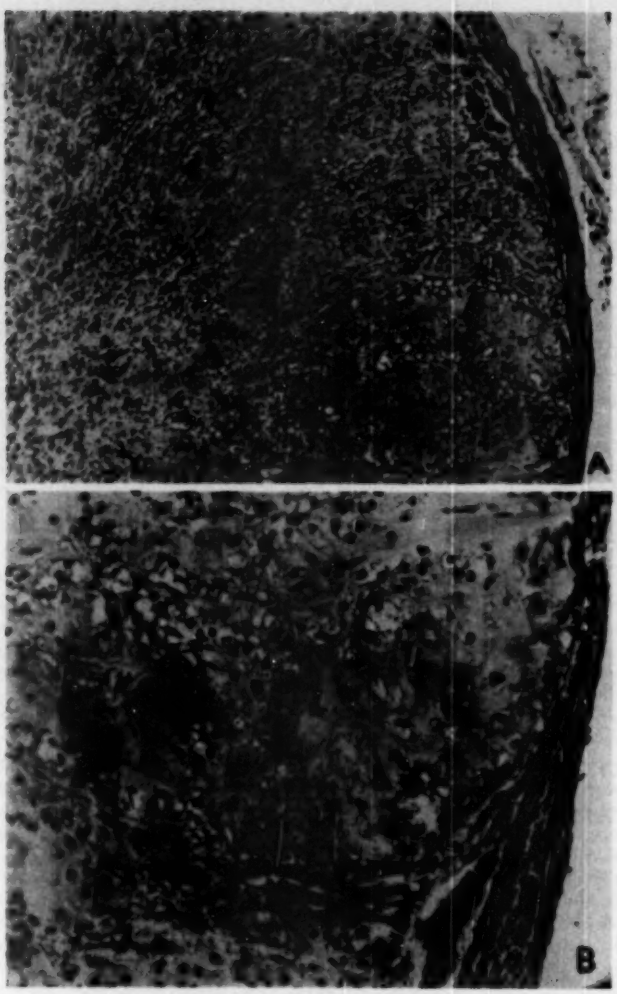

Fig. 1.—Amyloid infiltration of the adrenal cortex: In *A* compressed remnants of the zona glomerulosa appear immediately beneath the capsule; × 110. *B*, higher magnification of the field shown in *A*; × 230.

diarrhea was not controlled by any treatment, including use of streptomycin, which was administered for suspected tuberculous enteritis. In the last few weeks of life the patient frequently suffered from periods of syncope. During the last ten days of life there were nausea and vomiting, necessitating parenteral administration of fluids. During the last several months, the temperature had become subnormal despite active pulmonary disease, and the blood pressure was consistently around 80 systolic and 50 diastolic. On April 19, 1948 the patient died of circulatory collapse after having received continuous oxygen therapy for thirty-six hours.

Postmortem Observations.—Necropsy revealed advanced pulmonary tuberculosis with extensive cavitation of both upper lobes of the lungs and diffuse miliary involvement of other lung areas. There was grossly evident amyloid disease of the liver and the kidneys. The adrenal glands were normal in size and external

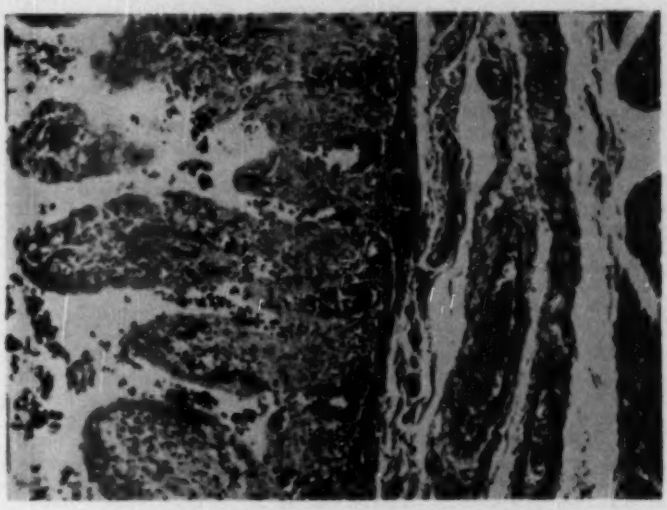

Fig. 2.—Amyloid infiltration of the small intestine; × 110.

appearance. On section a translucent, waxy, pale appearance of the cortex was noted. The glands had a firm rubbery consistency. The medullary areas were normal in appearance. The stomach and intestines were grossly normal.

Microscopic study revealed widespread pulmonary tuberculosis and amyloid disease of liver and kidneys. There was minimal amyloid involvement of the spleen.

The cortical tissue of both adrenal glands was extensively distorted and largely replaced by abundant amyloid deposits, which all but completely effaced the cords and cellular clusters of the fascicular and reticular zones (fig. 1). Broad perivascular expanses of amorphous amyloid impregnated and replaced parenchymatous cells with the result that a rather homogeneous mass obscured the normal structure. Small clusters of atrophic cortical cells of the zona glomerulosa persisted immediately beneath the capsule. There was obvious encroachment on these clusters by

spreading amyloid deposits. There was no histologic evidence of tuberculous involvement of the adrenal glands; cultures and animal inoculation of adrenal tissue were not done.

There was extensive diffuse deposition of amyloid which replaced most of the mucosal stroma of the intestine, compressing and destroying the majority of the epithelial crypts (fig. 2).

All of the essential findings necessary for the clinical diagnosis of Addison's disease were noted in this case. They were: (1) pigmentation of the skin and buccal membranes; (2) extreme asthenia, emaciation and syncopal attacks; (3) severe nausea and vomiting; (4) persistent hypotension, and (5) subnormal temperature in spite of active pulmonary tuberculosis. Of interest was the apparent relationship between the severe diarrhea and the intestinal amyloidosis. It appears logical to assume that the extreme amyloid involvement of the intestinal mucosa seriously interfered with absorption of fluids and other intestinal functions sufficient to account for this complaint.

SUMMARY

A case of Addison's disease resulting from extensive amyloid disease of the adrenal glands secondary to chronic pulmonary tuberculosis is presented.

DOUBLE ESOPHAGUS WITH CARCINOMA IN ONE

Report of a Case with Autopsy

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AND
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THE AVAILABLE literature reveals reports of only 3 cases of double esophagus. Cautley,[1] in a review of anomalies of the esophagus, mentioned cases reported by Blasius in 1674 and Kathe in 1907. A case of double esophagus and double stomach in a 17 day old girl was reported by Gjörup[2] in 1930. Gjörup referred to Kathe's case in his review of the literature and mentioned 1 other case, in both of which, he held, the double esophagi were false passages.

REPORT OF A CASE

A 49 year old white man first noted swelling of the left side of his neck in August 1947. This was followed in ten days by enlargement of the right side of the neck. A biopsy specimen taken at that time was diagnosed as lymphoepithelioma, and the patient entered a midwestern clinic in September 1947. His pharynx and larynx were carefully examined for a probable source of the neoplasm. Roentgen examination of the esophagus and stomach were reported as showing nothing of significance. The patient was given radiation therapy with no effect. He noted swelling of the right arm and enlargement of the right anterior chest wall. He returned to the clinic for further radiation therapy in March 1948 and May 1948. No improvement was noted. Difficulty in walking developed, owing to spasm of the leg muscles, and also the swelling of the right upper extremity progressed. A course of nitrogen mustard therapy in July 1948 resulted in improvement. This remission was brief, and on September 1 he applied for admission to this hospital for further nitrogen mustard therapy. The patient mentioned no complaints that could be referred to the gastrointestinal system. His past history revealed that in 1936 he was hospitalized for six months on account of tuberculosis and was then discharged as an "arrested case."

Physical examination revealed a tall, thin, chronically ill white man, who kept his right leg flexed, owing to spasm of the sartorius muscle. There were marked swelling, hardness and redness of the skin of the neck. Perforation of the nasal septum was noted. The right upper extremity was swollen. The anterior wall

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Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. Cautley, E.: Brit. J. Child. Dis. **14**:1, 1917.
2. Gjörup, E.: Acta pædiat. **15**:90, 1933.

of the right half of the chest was edematous, hot, indurated, and the patient stated that it itched. Marked wasting of the leg muscles was present. A hard mass was felt in the left axillary region.

The blood count and the urinalysis gave normal results. A roentgenogram of the chest demonstrated an infiltration at the right eighth anterior interspace. A metastatic survey revealed cystic areas surrounded by areas of increased bone density in the head of the right humerus.

Biopsy of the mass in the left axillary region and of the anterior wall of the chest revealed changes which were thought to be compatible with Hodgkin's dis-

Fig. 1.—The stomach and the lower parts of the esophagi opened along the lesser curvature and the anterior esophageal wall. Markers indicate the opening of the posterior esophagus and the anterior cardioesophageal junction.

ease. The patient was given a course of nitrogen mustard therapy with no improvement. Subsequently he received radiation therapy to the neck and chest without benefit. His previous biopsy specimen was obtained and interpreted by the pathologist as undifferentiated metastatic carcinoma. The patient began to have difficulty in swallowing; he frequently had thoracic pain, and his condition rapidly deteriorated. He died on Nov. 12, 1948.

At autopsy a nodular tumor involved the mediastinal structures surrounding the lower part of the trachea, the arch of the aorta and the middle third of the esophagus. The mediastinal structures were removed *en masse* from a point 5 cm.

above the tracheal bifurcation. The trachea and the aorta, although surrounded by the tumor, showed no gross evidence of tumor invasion. A posterior transverse section revealed the esophageal wall practically replaced by gray, friable tumor, and similar tissue, in a seminecrotic state, filled the lumen.

When microscopic sections were examined it was discovered for the first time that there were two esophageal lumens, the more anterior one lined by normal

Fig. 2.—Double esophagus. Markers indicate the lumens of the esophagi. The bifurcation of the trachea and a portion of the aortic arch may be seen.

epithelium except for small areas of tumor invasion and the posterior one lined entirely by cancer cells except for small areas of stratified squamous epithelium. Reexamination of the gross specimen revealed the two lumens (figs. 1 and 2). The anterior one was normally patent and opened into the stomach in a normal anatomic relationship. The posterior lumen was filled with seminecrotic, friable gray tissue which invaded and almost replaced the muscular wall in some areas. This esophagus extended caudally along the greater curvature of the stomach to open 6 cm. distal

to the opening of the normal anterior esophagus. This gastric opening was small (2 mm.) and was concealed by an overlapping fold of gastric mucosa. Since this anatomic anomaly, obscured by the mediastinal portion of the tumor, was not recognized until microscopic sections were examined, the exact limit of the anomalous organ could not be ascertained. Clinical and radiologic examinations of the pharynx and esophagus had not revealed the presence of the posterior organ.

The posterior esophagus was grossly involved by tumor down to a point 2 cm. from its opening into the stomach. The upper limit of involvement could not be determined since this extended beyond the upper limit of the gross specimen removed at necropsy.

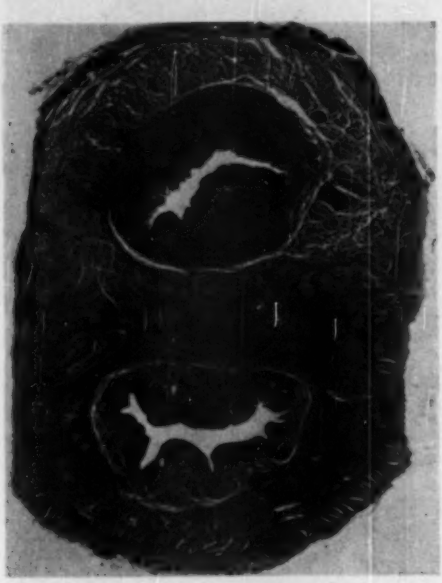

Fig. 3.—Stained slide (× 4). The anterior esophagus has an almost complete epithelial lining. Contrast the arrangement of the muscle layers in the two esophagi.

Microscopically, both esophagi had thick, smooth muscle walls which blended indistinguishably. The muscle layers of the posterior esophagus were indistinct but were roughly divided into inner longitudinal and outer circular layers in contrast to the anterior esophagus, which had anatomically normal inner circular and outer longitudinal layers (fig. 3). Skeletal muscle fibers were mixed with the smooth muscle in sections from the middle third.

The anterior esophagus was lined by stratified squamous epithelium, and histologically normal mucous glands were found in the submucosa of both. The posterior esophagus was lined by papillary adenocarcinoma and small areas of stratified squamous epithelium, as described (fig. 4 *A*). The lumen was filled with carcinoma, mucus and inflammatory exudate. The cancer was fairly well differen-

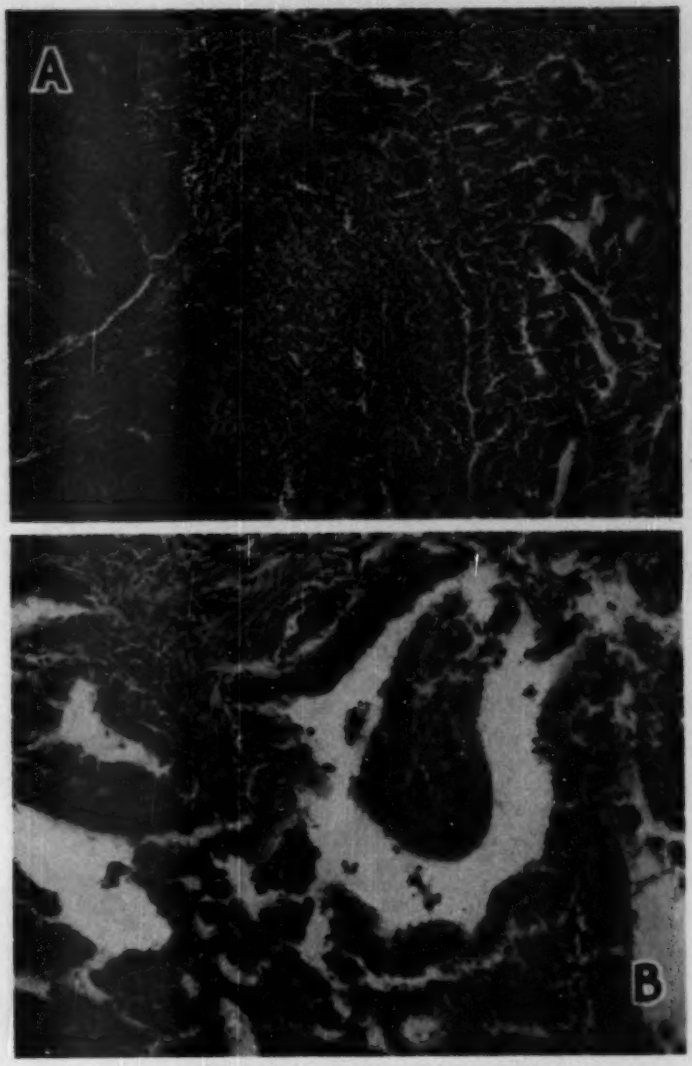

Fig. 4.—*A*, wall of the posterior esophagus (× 100). *B*, wall of the posterior esophagus showing cancer (× 200).

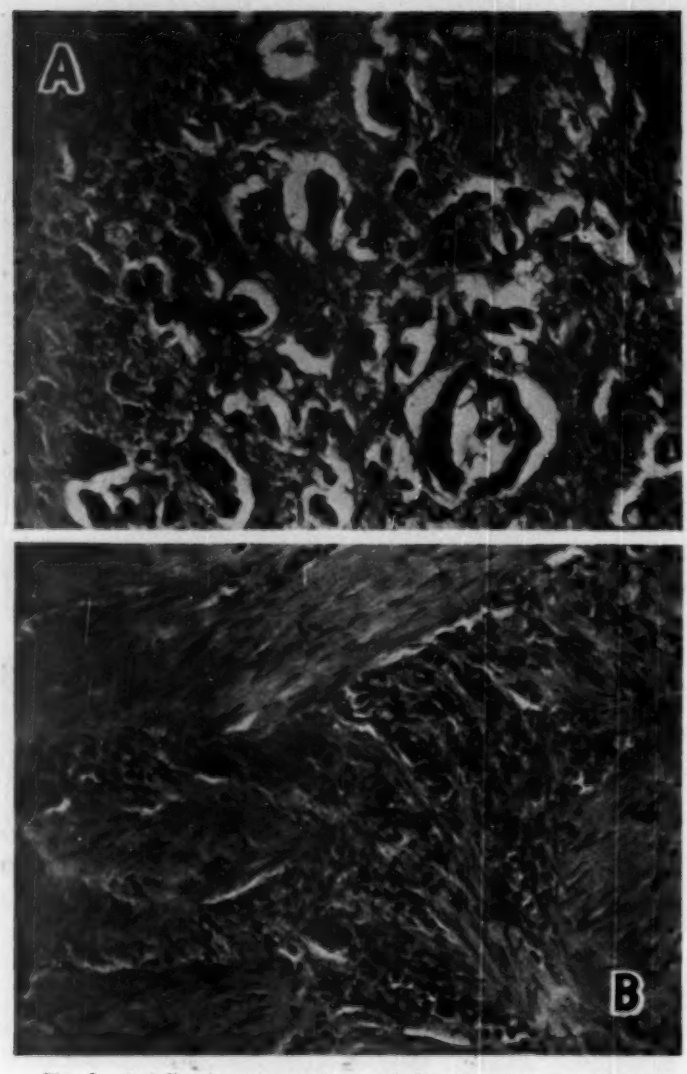

Fig. 5.—*A*, infiltrating adenocarcinoma of the submucosa of the posterior esophagus (× 200). *B*, anaplastic carcinoma infiltrating the muscular wall of the esophagus (× 200).

tiated near the lumen into secreting glands lined by columnar epithelium (fig. 4 *B*). In the deeper tissue it became less differentiated, showing a tendency to acinous grouping or forming glands of irregular size and shape (fig. 5 *A*). Anaplastic groups of small, darkly staining tumor cells infiltrated the muscular coats of both esophagi (fig. 5 *B*) and invaded the mucosa and epithelium of the anterior esophagus at one point. The most extensive invasion of the muscular wall occurred at the upper end of the specimen.

The metastatic tumor was largely anaplastic, but some groups of cells had acinous or glandular arrangement. Metastatic tumor invaded the lymphatic channels and tissue of the skin of the chest wall, the pectoralis muscle, the thyroid gland, the lungs, the stomach wall, the head of the pancreas and lymph nodes. No metastatic tumor was found in other organs. An incidental finding was multiple carcinoid of the ileum with local metastases.

COMMENT

The main tumor involved the anomalous esophagus and could be traced through the muscle layers to the surrounding mediastinal structures. The differentiation of the tumor cells decreased centrifugally from the esophageal lumen, and very anaplastic cells infiltrated the lymphatic channels as described. Review of the previous surgical biopsy material showed cells of the same type as were present in the more anaplastic areas of the main tumor. No evidence of Hodgkins' disease or lymphoepithelioma could be demonstrated in necropsy material. The findings of an anomalous organ, a main tumor involving this organ with decrease in differentiation of tumor tissue in surrounding structures and an absence of any neoplastic process other than an incidental carcinoid of the ileum substantiate the opinion that this was a primary carcinoma arising in an anomalous esophagus.

SUMMARY

A case of double esophagus is presented. The patient did not have the usual symptoms associated with carcinoma of this organ because one esophagus continued to function. He did have dysphagia terminally.

MYXOMA OF THE HEART (LEFT AURICLE)

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NEW ORLEANS

PRIMARY tumors of the heart are uncommon. Leach [1] found 1 primary neoplasm of the heart (rhabdomyosarcoma) in 6,275 autopsies. Lymburner [2] found 4 primary tumors of the heart in 8,500 autopsies, of which 2 were myxoma, 1 rhabdomyoma and 1 angioma. The present case should be placed on record because of the infrequent occurrence of myxoma of the heart and because of the large size of the neoplasm in this instance.

REPORT OF A CASE

S. C., a 68 year old Mexican woman, had been under treatment and observation for twelve years in the department of ophthalmology. On Aug. 22, 1949, she entered Jefferson Davis Hospital. Two weeks previously, according to her daughter, she had begun to be short of breath, and this had gradually become worse, so that she now had to sit up to breathe and frequently awoke at night short of breath. Swelling of her feet, legs and hands had gradually developed. She had had frequent frontal headaches and occasional dizzy spells. She had had a nonproductive cough for one month. She had complained of "pain in the heart" for at least twenty years. There was no history suggesting rheumatic fever.

She was a well developed, well nourished Mexican woman, appearing to be of about the stated age, and seeming moderately short of breath. Her oral temperature was 99 F.; her respiratory rate, 26 per minute; her blood pressure, 140 mm. of mercury systolic and 90 diastolic. Her heart appeared to be large. The left border of the heart was percussed in the anterior axillary line; the right border was substernal. The heart rate was 88 per minute without pulse deficit; the rhythm was irregular, with an occasional premature ventricular contraction. There was a loud, harsh blowing systolic murmur with accompanying thrill over the mitral area. This murmur was transmitted to the axilla. Harsh moist rales were heard over both lung fields, especially at the bases posteriorly. The legs were edematous, as were her hands. The abdomen was distended, and a fluid wave could be elicited.

She was digitalized and restricted to a low sodium diet. Mercurial diuretics were administered at intervals. On August 31 an electrocardiographic tracing was interpreted as showing auricular fibrillation and myocardial damage or digitalis effect or both (cupped S-T segment with diphasic T wave). On September 6 a

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1. Leach, W. B.: Arch. Path. **44**:198, 1947.

2. Lymburner, R. M.: Canad. M. A. J. **30**:368, 1934.

teleoroentgenogram showed moderate enlargement of the cardiac silhouette, with prominence of the pulmonary artery. She improved rapidly with treatment and on September 22 was discharged to return to the outpatient clinic.

On October 23 she returned to the emergency room. Her history was again obtained from her daughter. She had cried out in her sleep during the night of October 22 and awakened unable to speak intelligibly, with her face drawn to the left. She was unable to move her arms or legs, but after about eight hours she could move her left arm and leg. Inability to move the right arm and leg persisted. The emergency room record states that the pulse rate at this time was 124 per minute and that the blood pressure was 150 systolic and 80 diastolic. The apex impulse was in the midclavicular line in the sixth interspace. No mention was made of either the presence or the absence of a murmur.

Digitalis was administered, and the patient was observed in the hospital for two days. She was then dismissed to the clinic as improved, her pulse rate at the time of dismissal being 108 per minute and the rhythm irregular.

On October 27 she returned to the clinic. Physical examination at this time showed the heart to be enlarged, the rate 64 per minute without deficit and the rhythm irregular. There was a grade 3 to 4 systolic murmur at the apex with associated thrill, also a low-pitched early diastolic murmur at the mitral area. There was flaccid paralysis of the right arm and leg, and the patient seemed stuporous. A few hours after admission, reexamination revealed no diastolic murmur. Despite treatment with digitalis, diuretics and aminophylline, and general supportive treatment, her condition gradually became worse, the edema and ascites increasing, and the stupor deepening until, on November 5, she died.

NECROPSY

Heart.—The measurements of the valves were as follows: tricuspid, 11 cm.; pulmonary, 7 cm.; mitral, 11 cm.; aortic, 7 cm. The valves were all delicate and appear capable of normal function. The wall of the left ventricle was 1.3 cm. in thickness; the wall of the right ventricle, 0.5 cm. in thickness. The myocardium was not remarkable in appearance. The intima was smooth and glistening in all cardiac chambers. With the exception of the left atrium, the cardiac chambers were not of unusual size. Attached to the interauricular septum over the fossa ovalis there was a smooth-surfaced oval tumor, 8.5 by 7 by 5.5 cm. in size, filling and distending the left atrium. The area of attachment was 1.8 cm. in diameter. The inferior aspect of this tumor appeared to fill and completely occlude the atrioventricular opening in the area of the line of attachment of the mitral valve, so that it was difficult to see how more than a very small amount of blood could pass. Other aspects of the tumor were closely approximated to the opening of the pulmonary veins, but in this respect the passage of blood would not have been so largely interfered with, since the blood flow was in the direction of the tumor. The tumor was mottled red, yellow and pink on surface examination and on sectioning. It was moderately soft and elastic but not friable. The heart and tumor together weighed 520 Gm. Two hundred and fifty grams of this weight was accounted for by the weight of the tumor.

Lungs.—Each lung weighed 600 Gm. The lower lobes of both lungs were reddened, heavy and non-air-bearing. There was an embolus 3 by 0.5 cm. in size in the pulmonary artery branch supplying the dorsal segment of the lower lobe of the left lung. (The origin of this embolus was not determined; permission to open the leg veins was not granted. No thrombi were noted in the common iliac veins.) This segment was slightly firmer but not redder than the remainder of the lobe. There was a considerable amount of green-stained material in the bronchi.

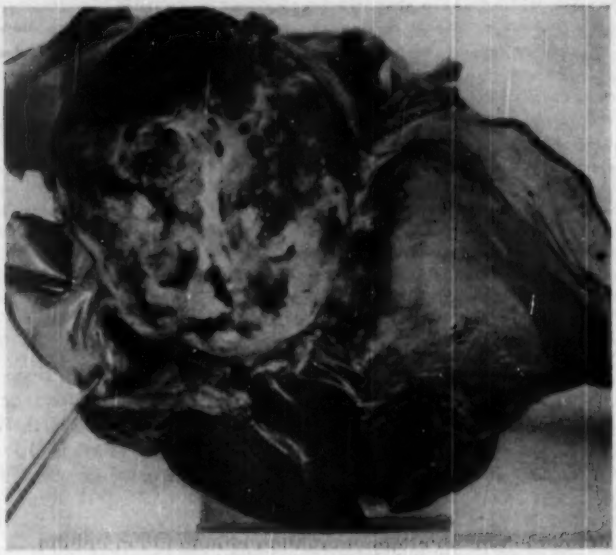

Fig. 1.—Tumor revealed by opening and spreading apart the left atrium. Note the smooth, shiny surface and mottled appearance. The point of attachment cannot be seen in this view. The scale shows inches.

Brain.—No areas of softening or hemorrhage were noted. No thrombi or emboli were seen. The meninges were delicate.

Other Organs.—These presented no relevant positive changes.

MICROSCOPIC EXAMINATION

Heart.—Sections of the tumor revealed a smooth surface covered in part by flattened, elongated cells similar to those lining serous surfaces. The main mass had a bluish pink myxoid intercellular substance with cells widely separated. The matrix was almost homogeneous in appearance; fine granules and occasional vacuoles were noted. There were

present spindle cells resembling plump young fibrous connective tissue cells with processes; also present were rounded cells with slightly eccentric nuclei and a few with stellate cytoplasm. All these had the appearance of fibrocytes in various stages of development. Occasional microscopic fields revealed numerous cells with round or slightly oval nuclei and little visible cytoplasm. Also seen were scattered polymorphonuclear neutrophilic cells of the granulocytic series and plasma cells. The blood vessels were sparse, dilated and filled with blood. A small amount of recent hemorrhage was present in areas. Scattered through the stroma were small areas of calcification. Beginning calcium deposition was seen in streaks and strands, as if along cell processes. The

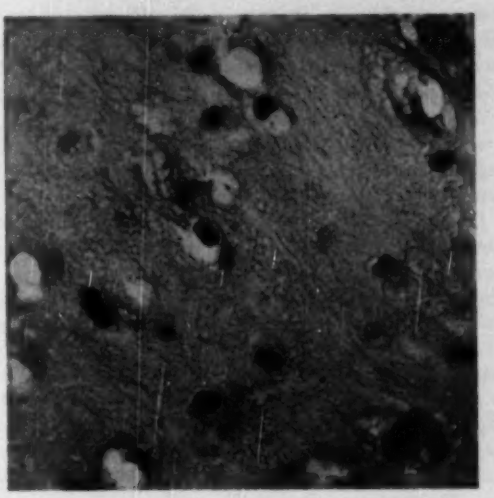

Fig. 2.—Photomicograph illustrating the myxoid intercellular substance with infrequent round cells. In other areas stellate and fusiform cells are present. Hematoxylin and eosin; × 440.

heart muscle fibers were large near the site of attachment, and there was moderate fibrosis on the inner aspect, fibrous tissue extending a distance into cardiac muscle in places. Immediately adjacent were seen mature fibrous connective tissue, numerous dilated vascular spaces and uniform capillaries projecting into the attached mass. This fibrous tissue diminished gradually as it extended into the tumor, and finally disappeared. There was also present considerable recent hemorrhage. Collections of lymphocytes and plasma cells were prominent near the junction of cardiac muscle and tumor. The greatest amount of calcification was noted in this zonal area.

Lungs.—Sections from the dorsal segment of the lower lobe of the left lung showed that the alveoli were partially collapsed, and many of them were filled with an exudate consisting primarily of erythrocytes. Sections from other parts of the lower lobes showed the alveoli to be completely filled with an exudate consisting primarily of polymorphonuclear leukocytes with a small amount of fibrin in threads. The alveolar walls were thickened, and cells suggesting an epithelial lining of the alveolar walls were present in many areas.

COMMENT

Cardiac myxoma is the most common, as well as the most debated, of the primary neoplasms of the heart. Thorel [3] was of the opinion that tumors of this type were merely organized thrombi. Many pathologists concur in this view. Ewing, on the other hand, considered that in the vast majority of reported cases myxoma of the heart was a genuine tumor. Willis [5] agreed with this opinion.

It is relevant to note that such myxomatous changes do not occur in organizing thrombi in other locations and that in the reported cases of ball thrombi of the auricles, no mention is made of any myxomatous change. On the other hand, the most frequent site for tumors of this type is the left auricle, and this is also true of free [6] or pedunculated [7] ball thrombi. The final decision will probably have to await adequate examination of myxomatous tumors of varying sizes and of unattached ball thrombi, since there can be little question that the latter are not neoplastic.

For many years these tumors were pathologic curiosities. The fact that they were rarely diagnosed before death was of no moment from any standpoint other than academic, since nothing could be done therapeutically. However, Beck [8] in 1942 and de Bakey [9] more recently stated that it is within the realm of possibility to remove such tumors surgically. These tumors are usually misdiagnosed as mitral stenosis and regurgitation, as was true in the present instance. Embolic phe-

3. Thorel, C.: Ergebn. d. allg. Path. u. path. Anat. **2**:455, 1910; **11**:90, 1915, quoted by Field, M. H.; Donovan, M. A., and Simon, H.: Am. Heart J. **30**:230, 1945.

4. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928, p. 188.

5. Willis, R. A.: Pathology of Tumors, St. Louis, C. V. Mosby Company, 1948, p. 654.

6. Wright, I. S.; Flynn, J. F., and Druet, K. L.: Am. Heart J. **27**:858, 1944.

7. Garvin, C. F.: Am. Heart J. **21**:371, 1941.

8. Beck, C. S.: Ann. Surg. **116**:161, 1942.

9. de Bakey, M.: Personal communication to the author.

nomena are common,[10] and in this case the cerebral symptoms suggested embolism, although none was found at necropsy. The changing character of the murmurs may be significant, although this was not appreciated in the present case because different examiners made the observations. Failure to respond to treatment is supposed to be a valuable diagnostic hint,[11] but this patient responded to treatment on first admission. Severe cardiac diseases in which the signs cannot be correlated with the usual etiologic types should suggest the possibility of a cardiac neoplasm, especially if the dyspnea is out of proportion to the physical findings.

SUMMARY

"Myxoma" of the heart of unusually large size is reported, with clinical and pathologic observations. Attention is directed to the possibility that such tumors may be surgically removed if recognized during life.

10. Young, R. D., and Hunter, W. C.: Arch. Path. **43**:86, 1947.
11. Ravid, J. M., and Sachs, J.: Am. Heart J. **26**:385, 1943.

Notes and News

Histochemical Society.—The first meeting of the newly organized Histochemical Society was held at the University of Pennsylvania Medical School in Philadelphia on March 24 and 25, 1950. The following officers were elected: President, Dr. G. B. Wislocki, of Harvard; Vice President, Dr. D. Glick, of Minnesota; Secretary, Dr. R. D. Lillie, National Institutes of Health; Treasurer, Dr. E. W. Dempsey, of Harvard; Councillors: Dr. O. Lowry, Washington University, St. Louis; Dr. G. Gomori and Dr. I. Gersh, University of Chicago; Dr. S. Mudd, University of Pennsylvania; Dr. A. Seligman, Beth Israel Hospital, Boston; Dr. J. F. A. McManus, University of Alabama; Dr. C. P. Leblond, McGill University, and Dr. J. W. Bartholemew, University of Southern California.

Thirty scientific papers were presented, and very active discussion ensued. Over two hundred scientists from various points in the United States, Canada and Latin America were registered, and about one hundred applications for original membership have been received.

Revised Edition of Motion Picture Reviews Now Available.—The Committee on Medical Motion Pictures of the American Medical Association has completed the second revised edition of the booklet entitled "Reviews of Medical Motion Pictures." This booklet now contains 225 reviews of medical and health films reviewed in *The Journal of the American Medical Association* to Jan. 1, 1950. Each film has been indexed according to subject matter. The purpose of these reviews is to provide a brief description and an evaluation of motion pictures which are available to the medical profession. Each film is reviewed by competent authorities and every effort has been made to publish frank, unbiased comments. Copies are available at a cost of 25 cents each from the ORDER DEPARTMENT, AMERICAN MEDICAL ASSOCIATION, 535 North Dearborn Street, Chicago 10, Ill.

International Cancer Congress.—The Fifth International Cancer Congress will be held in Paris, France, July 15-22. Dr. A. Lacassagne, Institute du Radium, Rue d'Ulm 26, Paris (5e), France, is the president of the congress, which is sponsored by the Union Internationale contre le Cancer. Edmund V. Cowdry, Ph.D., Washington University School of Medicine, St. Louis 10, Mo., is the vice president of the Union for the United States. William U. Gardner, Ph.D., Yale University School of Medicine, New Haven, Conn., is a member of the program committee and will receive titles and abstracts of papers to be presented from the United States. No invitation to the congress is required. Those expecting to attend should promptly notify the Union, pay a small registration fee and request reservation of the hotel accommodations desired.

Deaths.—Aubray Crafton Gose, of Memphis, Tenn., formerly assistant professor of pathology at the University of Tennessee, died in Hughesville, Ark., February 8, aged 37, of an accidental gunshot wound. He was a specialist certified by the American Board of Pathology and a member of the College of American Pathologists and the American Society of Clinical Pathologists.

Howard Nelson Kingsford, professor emeritus of pathology and bacteriology at the Dartmouth Medical School, Hanover, N. H., died February 9, aged 78, of carcinoma of the prostate with metastases.

Herbert Dodge Pease, of Richmond Hill, N. Y., died in Springfield, Mass., February 14, aged 79, of cerebral thrombosis and general arteriosclerosis. He was bacteriologist for the New York State Pathological Laboratory in Buffalo from 1898 to 1900; instructor in bacteriology at the Sheffield Scientific School, Yale, in 1900; director of the antitoxin hygienic laboratory, State Department of Health of New York from 1901 to 1909, and director of the Lederle Laboratories from 1909 to 1918. He was a member of the American Association of Pathologists and Bacteriologists and the Society of American Bacteriologists.

Books Received

CLINICAL PATHOLOGY: APPLICATION AND INTERPRETATION. By Benjamin B. Wells, M.D., Ph.D., professor of medicine, University of Arkansas School of Medicine. Pp. 397, with 32 figures and 38 tables. Price $6. W. B. Saunders Company, W. Washington Sq., Philadelphia; 7 Grape St., Shaftesbury Ave., London, W.C.2, England, 1950.

This volume is written for clinicians and clinical pathologists. The practitioner of medicine, says the author, "must know at least three things about every laboratory procedure he expects to use: (1) when to use it, (2) how to interpret the results and (3) what technical or physiologic limitations must be taken into account in the interpretation."

Dr. Wells discusses diseases of the different body systems from the standpoint of the laboratory tests that might be of value in their diagnosis or in understanding their course and nature. The relevance of various laboratory procedures, the interpretation of the results and their significance are well handled. Precautions to be taken in the performance of certain tests are brought out, but the book does not deal with technic. It is an interpretative work that can be highly recommended to alert practitioners and to clinical pathologists.

GRUNDLAGEN DER FUNKTIONELLEN UROLOGISCHEN RÖNTGENDIAGNOSTIK. By Walter Pfeifer, M.D., Roentgen Institute of Leipzig University and Roentgen Institute of Oldenberger Hospital, Sanderbusch. Pp. 88, with 48 illustrations. Price 9.60 German marks. Georg Thieme, Diemershaldenstrasse 47, Stuttgart-O, Germany, 1949.

As indicated in the title, this volume presents the functional examination of the kidney as accomplished by means of contrast mediums and roentgen ray. It is not intended that it be a treatise on urologic radiology, and indeed it is largely experimental. The subject is well illustrated with numerous, adequate examples. To those concerned particularly with renal disease, this book offers some fresh vistas.

CONGENITAL ANOMALIES OF THE HEART AND GREAT VESSELS: CLINICOPATHOLOGIC STUDY OF 132 CASES. By Thomas J. Dry, Robert L. Parker, H. Milton Rogers, Jesse E. Edwards, Howard B. Burchell, Arthur H. Bulbulian, of the Mayo Clinic. Publication 47, American Lecture Series. A Monograph of American Lectures in Pathology. Edited by Paul R. Cannon, professor of pathology at the University of Chicago School of Medicine. Pp. 67, with 80 illustrations. Price, $4.50. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1949.

This monograph is actually an atlas of sixteen main types of congenital anomalies of the heart and great vessels observed in the clinicopathologic material of the Mayo Clinic. Each anomaly is beautifully and clearly illustrated with colored models, photographs, diagrams, roentgenograms and electrocardiographic tracings, each accompanied by a brief description of the pathologic features. Brief biographic sketches of outstanding physicians who have contributed to knowledge in this field are included. Because of Spitzer's basic contribution to the understanding of cardiac anomalies one would have liked to see his name mentioned.

This book is an excellent example of how to make available to members of the medical profession material which was primarily assembled for an exhibit.

Because of the clear and straightforward presentation of a rather complicated subject, it should prove useful to students and physicians as an introduction to the study of congenital cardiovascular diseases. Obviously, it does not intend to and cannot replace the more complete works on the subject, such as Abbott's atlas and Taussig's recent book.

As Dr. Willius states in his foreword, the recent amazing advances in cardiovascular surgery, particularly in the surgery of congenital anomalies of the heart and great vessels, have renewed interest in this field and made this a most opportune time for the publication of a work of this type.

HEMOLYTIC DISEASE OF THE NEWBORN. By M. M. Pickles, D.M., Nuffield graduate assistant in clinical pathology at Radcliffe Infirmary, Oxford. Pp. 181, with 21 illustrations and 19 tables. Price $4.50. Charles C Thomas, Publisher, 307-327 E. Lawrence Ave., Springfield, Ill., 1949.

A voluminous and highly controversial literature has arisen around the subject of isoimmunization and hemolytic disease of the newborn since the Rh factor was discovered some years ago. Involved speculations on serologic matters unsupported by experimental fact and glamorized by vacuous rhetoric and confusing nomenclature have tended to complicate the problem for the physician. Poorly informed lay writers have promoted a great deal of unnecessary anxiety among expectant mothers and have thus intensified the plight of the physician. It is refreshing, therefore, to find a work whose purpose is to present known and proved facts and critically survey the recent knowledge concerning this important subject. The statements made in the book are well documented by fact and reflect a mature working knowledge of the clinical as well as of the serologic aspects. The subject material is well organized into chapters on the historical evolution of the disease, an excellent discussion of Rh antigens and antibodies and their relation to hemolytic disease, the mechanism of immunization and the clinical patterns of the disease, a concise but comprehensive chapter on general and ancillary treatment and finally a summary of practical points in relation to hemolytic disease and some of the sequelae and other suggested manifestations of Rh incompatibility. The monograph is concise, complete and authoritative. It will be valuable to the clinician, especially the obstetrician and the pediatrician, as well as to the serologist and the hematologist.

SCHILDDRÜSE UND BASEDOW. By Prof. Dr. med. Wilhelm Eickhoff. Pp. 127, with 42 illustrations and 18 tables. Price 10.80 German marks. Georg Thieme, Diemershaldenstrasse 47, Stuttgart-O, 1949.

CLINICAL ELECTROENCEPHALOGRAPHY. By Robert Cohn, electroencephalographer and director of neurological research, United States Naval Hospital, Bethesda, Md. Pp. 639, with 17 illustrations and numerous exemplary tracings. Price $14. McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 18; Aldwych House, Aldwych, London, W.C.2, 1949.

COCCIDIOSIS. By Sterling Brackett and 42 other authors. Pp. 195. Price $3. Annals of the New York Academy of Sciences, volume 52, art. 4, Nov. 10, 1949.

VITAMIN E. By Karl E. Mason and 116 other authors. Pp. 365. Annals of the New York Academy of Sciences, volume 52, art. 3, Oct. 31, 1949. Price $4.50.

DAS CHRONISCHE CYSTISCHE HYDROM DER DURA IN SEINEN BEZIEHUNGEN ZUM SOG. CHRONISCHEN TRAUMATISCHEN SUBDURALEN HÄMATOM UND ZUR PACHYMENINGITIS HÄMORRHAGICA INTERNA IM LICHTE DER RELATIONSPATHOLOGIE. By Alfred Bannwarth, M.D., Dozent Dr. med. habil. Munich, Germany. Pp. 57, with 19 illustrations. Price 9.60 German marks. Georg Thieme, Diemershaldenstrasse 47, Stuttgart-O, Germany, 1949.

This monograph represents an attempt to refer chronic subdural hematoma, pachymeningitis hemorrhagica interna and cystic hydroma of the dura to a common pathogenesis. The clinical material presented in the first half of the work does not abet the argument, and the anatomic evidence detailed later is obtained from other sources. What is new is the author's deductions from these data.

The view that these three conditions are similarly intradural in location requires more convincing documentation. That they result from different rates or degrees of hemorrhage, alone or with transudation, is as acceptable a hypothesis as has been offered. When there is a high concentration of protein in the hydroma fluid, one cannot readily imagine that the fluid originates from the cerebrospinal fluid, and the conclusion of the author seems justified.

His thesis is clearly set forth and is accompanied by an adequate bibliography.

GREEN'S MANUAL OF PATHOLOGY. By H. W. C. Vines, M.A., M.D., professor of pathology, University of London, and dean, Charing Cross Hospital Medical School. Seventeenth edition. Pp. 1,200, with 730 illustrations. Price $8. Williams & Wilkins Co., Mt. Royal & Guilford Aves., Baltimore 2, 1949.

This is a textbook, written primarily for medical students who are beginning the study of human pathology. The book is divided into two parts. The first part is concerned with a general discussion of pathologic processes. The second part describes the effects of these processes in different systems and organs. The contents of the text follow the pattern customarily used in elementary textbooks of pathology. Emphasis is placed on gross and microscopic pathology, but there are many appropriate comments on clinical, physiologic and chemical aspects of important diseases. In general, the descriptive illustrations of the gross and microscopic appearance of lesions are well chosen, but an occasional one is not very clear. The author has written in an attractive style and has used new terminology only after concise definition of terms. Apparently, he has not expected the student to seek information other than that offered in the text, for no bibliographic references are given. There is little to choose between this book and many books written by others for the same purpose. It has few distinguished characteristics which would make it a valuable adjunct to the teaching of the relations between classic pathology and abnormal physiology or chemistry. Textbooks for making this correlation are badly needed as the methods and interests of the basic medical sciences become more intimately related.

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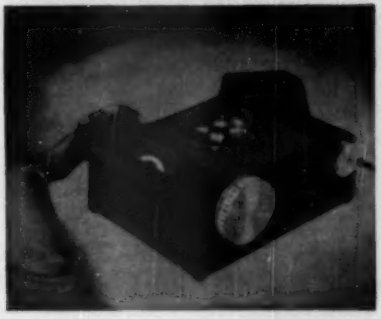

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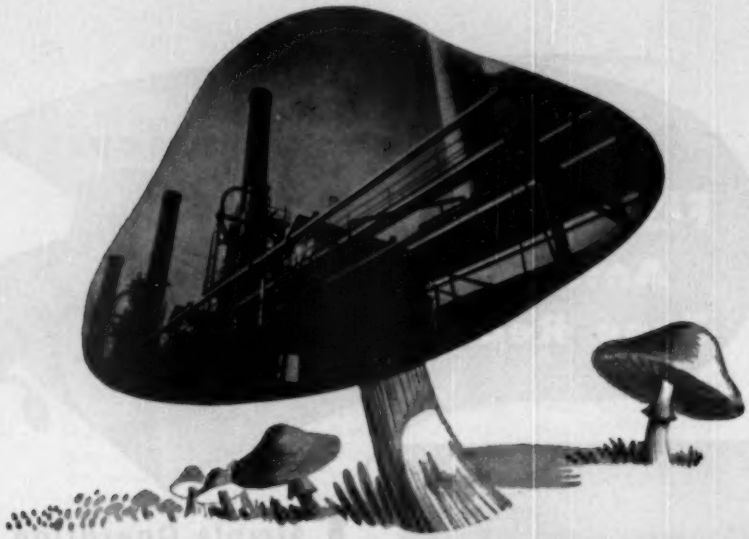

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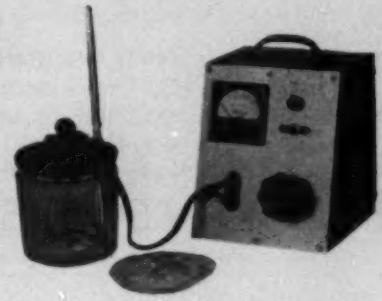

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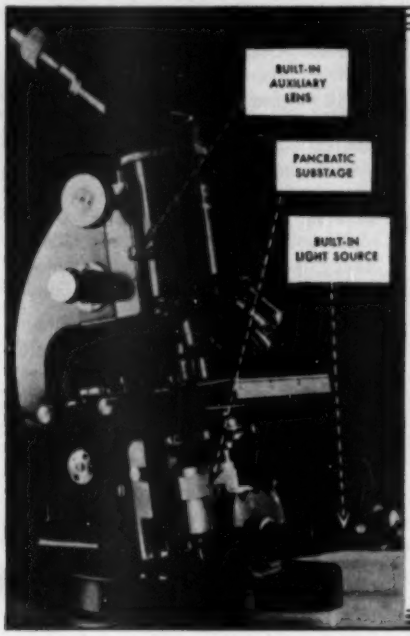

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